Thrombolysis in acute stroke under dual antiplatelet therapy: perspectives arising from translational studies

Franziska Lieschke, Yi Zheng, Christian Foerch, Klaus van Leyen*

We have recently established a mouse model of focal stroke under dual antiplatelet therapy (DAPT) to study tissue plasminogen activator (tPA)-associated hemorrhagic transformation. The purpose of this short perspective is to discuss the rationale for establishing the model, highlighting its relevance for addressing unresolved clinical questions. Hemorrhagic conversion of the ischemic stroke remains one of the major liabilities of thrombolytic therapy with tPA, contributing to unfavorable outcomes and failed regeneration. This was recognized early on, and the resulting restrictions on tPA usage have led to only a minor percentage of stroke patients receiving any kind of drug treatment to limit ischemic injury. Broadening the patient population eligible for thrombolytic therapy is a major goal, and thus efforts are being directed at optimally defining inclusion criteria based on prior drug treatment status, among other factors. DAPT with aspirin and clopidogrel (ASA + CPG) is commonly given to patients at high risk for atherothrombotic events. However, clinical data to date has not been entirely clear as to whether the increased likelihood of bleeding following thrombolysis in patients on DAPT is indeed detrimental to patient outcome, and many see this potential downside outweighed by the benefits of recanalization of the blocked vessel. Accordingly, current guidelines allow for tPA thrombolysis in patients under DAPT. Nonetheless, doubts remain if on balance tPA is actually beneficial in these patients, and these doubts may lead to undertreatment. Final clarity might be achieved with a prospective, randomized clinical trial, but it appears unlikely that this will ever occur. In this situation, modeling the process in animals subjected to experimental ischemic stroke under DAPT can provide insights into mechanisms of hemorrhagic transformation (HT). Even more importantly, establishing such a model enables researchers to test possible strategies to mitigate the bleeding risk in patients on DAPT. If the safety of tPA thrombolysis can be increased by reducing hemorrhage, this could clearly tilt the balance towards favoring tPA treatment, and thus improve long-term outcomes of ischemic strokes. Testing such an approach in the animal model is the best first step in evaluating the utility of such an adjuvant.

Current use of dual antiplatelet therapy: DAPT is used to prevent atherothrombotic events in high risk patients, for example in patients with acute coronary syndrome with or without percutaneous coronary intervention, as well as in percutaneous coronary intervention patients without acute cardiac symptoms. Aspirin is combined with a P2Y12 inhibitor such as Clopidogrel, Ticagrelor, or Prasugrel and should be continued for up to one year. Furthermore, DAPT is increasingly used as secondary prevention following acute ischemic stroke of atherothrombotic origin within the first 24 hours after stroke onset: Following high risk transient ischemic attack or minor stroke DAPT with preferably ASA + CPG is continued for 10 to 21 days. Another clinical scenario of relevance is DAPT in acute stroke patients with a tandem occlusion of the internal carotid artery and the middle cerebral artery. If those patients are subjected to endovascular treatment, stent implantation into the proximal internal carotid artery is necessary to allow passage with the retriever system. Here again, DAPT with ASA + CPG is indicated to prevent re-occlusion after stenting, although many of the patients have been treated with tPA just minutes before. Before, during and after artery stenting, it is recommended to continue the DAPT for 1–3 months.

Clinical studies on pre-stroke DAPT and increased HT following tPA thrombolysis: Multiple retrospective analyses on pre-stroke antiplatelet treatment and tPA thrombolysis in acute stroke patients indicated a similar long-term survival and functional outcome compared to patients without antiplatelet medical history, despite the significantly increased risk of symptomatic intracranial hemorrhage (sICH). In those studies, the hemorrhagic outcome was classified by both imaging characteristics and clinical presentation. As such, Diedler et al. (2010) analyzed the data of 11,865 patients collected in the Safe Implementation of Treatments in Stroke International Stroke Thrombolysis Register from 2002–2007, which included 151 patients on DAPT with ASA + CPG. While in their multivariable analysis, ASA + CPG was associated with increased risk for sICH, no significant differences in mortality and functional outcome at 3 months were found between patients on prior antiplatelet medication compared to antiplatelet naïve patients (Diedler et al., 2010). In contrast, a meta-analysis by Pan et al. (2015) including 11 studies with a total of 19,453 patients confirmed the increased sICH risk for patients on pre-stroke DAPT but claimed a trend toward reduced probability of good functional outcome in patients on pre-stroke antiplatelets (all types together), although this finding was not statistically significant. Another meta-analysis by Luo et al. (2016) included 19 studies and reported a confounder-adjusted positive association between pre-stroke antiplatelets (any kind) and sICH, with no differences in mortality and functional outcome. Their subgroup analysis revealed, that among all antiplatelets, patients on DAPT with ASA + CPG were at highest risk for sICH. Xian et al. (2016) performed an analysis on the data collected in the American Heart Association and American Stroke Association Get With the Guidelines Stroke registry including 2397 patients on prior DAPT with ASA + CPG, which replicated the increased odds of sICH for DAPT patients in unadjusted and risk adjusted models, but demonstrated a similar mortality and even better functional outcomes in these patients. Finally, Tsivgoulis et al. (2018) recently reported another retrospective analysis on the data collected in the Safe Implementation of Treatments in Stroke International Stroke Thrombolysis Register from 2010–2017 focusing on particularly DAPT. After balancing groups (antiplatelet naïve *versus* prior DAPT) for all baseline characteristics, sICH rates were significantly higher in DAPT patients, while 3-month outcomes (mortality and functional outcome) did not differ (Tsivgoulis et al., 2018). The accumulating data eventually led to the elimination of pre-stroke DAPT as a contraindication for tPA thrombolysis. However, there are potentially important long-term cognitive effects. Cerebral microbleeds have been linked to a decline in various cognitive and executive functions (Weekman et al., 2016; Moulin and Cordonnier, 2019). Although this question has to date not been conclusively studied for HT under DAPT, it stands to reason that the observed sICH may lead to even more severe cognitive effects. This type of long term brain injury is not captured in the aforementioned clinical outcome studies, which rely on assessments after several months and which often only asses the independency in daily activities (e.g., modified Rankin Scale) as a measure for the degree of disability. Cognitive functions such as attention, executive functioning, learning and memory, language and social cognition are usually not queried. Avoiding HT could thus increase the benefit of tPA in patients on pre-stroke DAPT both by tipping the balance clearly towards bigger benefits of tPA in the functional outcomes at 3 months, and by also reducing the risk of long term cognitive decline and dementia.

Experimental stroke model of DAPT-related HT: A prospective randomized clinical trial evaluating whether the benefit of tPA outweighs the increased risk of HT in patients on pre-stroke DAPT would contribute to final clarity, but is difficult to conceptualize, especially because tPA thrombolysis is now increasingly combined with mechanical thrombectomy. At this point, animal models can be interesting. Beyond the fact that rodent studies can be conducted in a controlled environment and under standardized conditions, there are two major reasons why establishing an animal model of DAPT-related HT is of value: 1) While the aforementioned clinical studies tell us something about the risks and benefits associated with thrombolysis under DAPT, they provide very little insight into mechanisms of HT. Here, animal

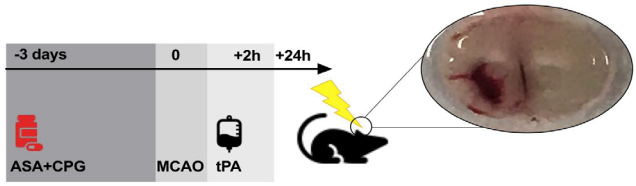

Figure 1 | **Model of HT treated with ASA + CPG in ischemic stroke.**
Mice were fed with ASA + CPG for 3 days, and subsequently subjected to transient MCAO for 2 hours. At the time point of reperfusion, tPA was infused intravenously if indicated. 24 hours after stroke onset, mice were sacrificed and outcome parameters, in particular brain hemorrhages were assessed. ASA + CPG: Aspirin and clopidogrel; HT: hemorrhagic transformation; MCAO: transient middle cerebral artery; tPA: tissue plasminogen activator.

studies provide a way forward, by allowing the researcher to study physiological and molecular correlates of brain injury. 2) Moreover and perhaps more importantly for the clinician, they provide a platform for testing novel ideas to reduce HT, by treating mice with possible neuroprotective or vasculoprotective therapies.

An extensive literature search did not reveal any existing animal models of pre-stroke DAPT in ischemic stroke. We therefore established an experimental stroke model of tPA treatment under DAPT in mice, successfully demonstrating increased levels of HT in mice treated with ASA + CPG prior to experimental stroke and thrombolytic therapy (Zheng et al., 2019). A major advantage of this model is the incorporation of FACS-based platelet activation measurements, allowing the platelet status of each individual mouse to be determined (Lieschke et al., 2020). For this, mice were fed with ASA + CPG diluted in their drinking water. After 3 days, blood was collected and tested *in vitro* using flow cytometry analysis of platelet activation markers. Mice were then subjected to 2 hours of middle cerebral artery occlusion followed by tPA infusion into the jugular vein. At 24 hours after the onset of ischemia, mice were sacrificed and the HT was assessed on brain sections (**Figure 1**). To our knowledge, this is the first study investigating the effects of DAPT in an experimental stroke model. We succeeded in effectively treating mice with ASA + CPG via drinking water, and achieved significant antithrombotic effects measured *in vitro* using flow cytometry. Furthermore, we demonstrated the feasibility to perform surgical procedures associated with the middle cerebral artery occlusion model inowing to the antithrombotic treatment-vulnerable and fragile tissue without increasing the amount of complications resulting from the surgical intervention itself. Our model can now be used to identify novel mechanisms of HT, and to develop strategies to reduce bleeding. Ultimately, the development of those strategies could further increase the benefit of tPA thrombolysis.

Ideally, adjunctive treatments could be used in a multimodal therapy in the future, maintaining the efficacy of pharmacological or mechanical thrombolysis. Possible candidates that stabilize the blood-brain barrier could be modulators of inflammation, as well as agents that reduce oxidative stress. In this context, inhibiting 12/15-lipoxygenase could be a promising approach, since this enzyme is upregulated in neurons and vascular endothelial cells in the peri-infarct region after experimental stroke. Both gene knockout and pharmacological inhibition of 12/15-lipoxygenase with agents including LOXBlock-1 or ML351 were shown to reduce HT in different contexts (Liu et al., 2017; Karatas et al., 2018). It remains to be seen whether the protection by this promising candidate will be confirmed in our mouse model of tPA thrombolysis under DAPT.

As with all models, there are limitations to consider. First, while DAPT in patients is most commonly initiated in the hospital following a first atherothrombotic event, the timepoint and duration of DAPT in animal models is set artificially not always perfectly reflecting the clinical scenario. Second, real patients, especially those on DAPT, more often suffer from several comorbidities that complicate the overall situation of the patient. Hemorrhagic conversion was found to typically occur in elderly patients (Diedler et al., 2010; Xian et al., 2016), whereas animals used in experimental studies are usually young and healthy and all experiments are conducted under controlled environmental and standardized conditions. Third, long-term outcome studies in animals are difficult. With most protocols, high mortality rates can be expected when extending the recovery period beyond several days.

To conclude, until neuro- and vasculoprotective candidates can be tested in clinical trials, animal models remain one helpful tool in establishing stroke treatment. Ultimately, these studies may lead to a safer application of tPA in a larger patient population than currently reached and may significantly improve the risk-benefit assessment in acute stroke treatment, eventually leading to improved long-term outcomes of ischemic strokes.

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