cima
CENTER FOR APPLIED MEDICAL RESEARCH
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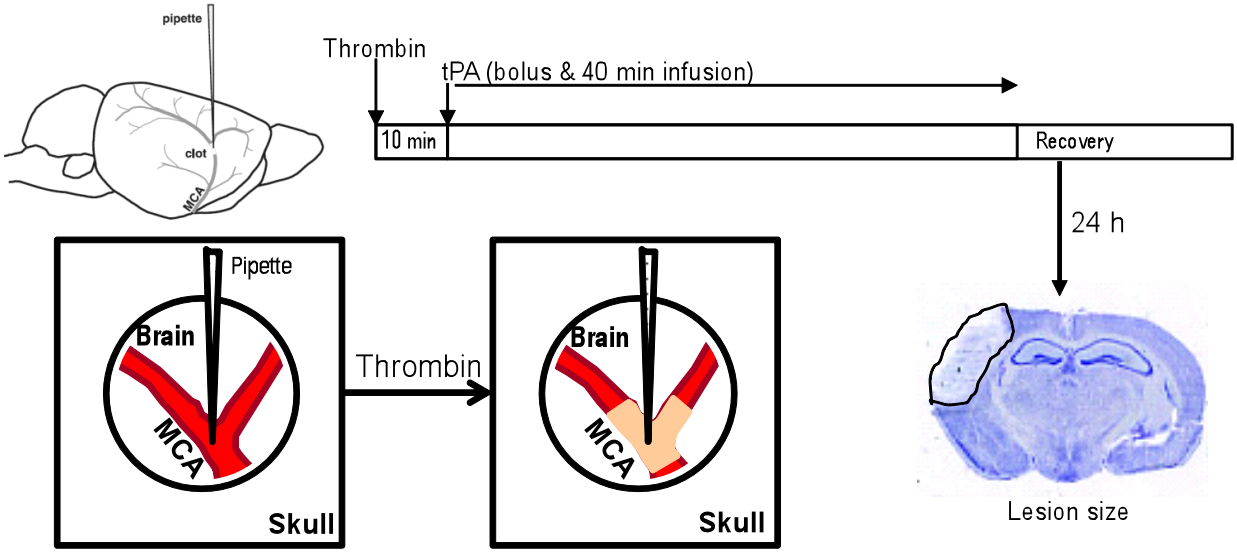
# Stroke: Animal models

Stroke, the second most common cause of death and major cause of disability worldwide (WHO 2015), can be caused either by a clot obstructing the flow of blood to the brain (called an **ischemic stroke**) or by a blood vessel rupturing and preventing blood flow to the brain (called a **hemorrhagic stroke**). Ischemic stroke accounts for 87% of all stroke cases. Intravenous recombinant tissue-type plasminogen activator (tPA), a thrombolytic agent, is the only pharmacological treatment globally approved for the treatment of acute ischemic stroke. However, due to its narrow therapeutic time window (3–4.5h) and the risk of promoting intracerebral haemorrhage (6.7%), tPA is given to a very limited number of patients. Moreover, only a third of treated patients have a good outcome, and increased risk of early death from intracranial haemorrhage (2%) has been reported. tPA has also shown neurotoxicity in experimental models of cerebral ischemia.

Testing potential acute therapies in animal models is presently the most common strategy for the development of new drugs for use in stroke. We perform a well established thromboembolic model of stroke in mice, which seems physiologically relevant and is now used by several groups to evaluate the effects of r-tPA either alone or in combination with putative neuroprotective drugs. However, in order to achieve translational success, preclinical models must take into account the existence of comorbidities frequently associated to stroke in humans.

✓ **Thromboembolic model of stroke in mice:** After anesthetizing the mice are with isoflurane, a small craniotomy is performed, the dura is excised and the middle cerebral artery is exposed. A glass micropipette is introduced into the lumen of the medial cerebral artery and 1 µL (1 UI) of murine α-thrombin is injected to induce a clot, in situ. To allow stabilization of the clot, the pipette is removed 10 minutes after the injection of thrombin. Rectal temperature is maintained at 37±0.5°C throughout the surgical procedure using a feedback-regulated heating system. Cerebral blood flow velocity, used as an index of the occlusion, is measured using laser Doppler within the middle cerebral artery territory during 60 to 120 minutes.




**Scheme of the murine model of thromboembolic stroke**.

✓ **Thrombolytic treatment:** Thrombolytic treatment is administered through a catheter inserted into the tail vein alone or with 200 µL of human recombinant tPA (r-tPA; Boeringher Ingelheim, Alteplase; 10% in bolus, 90% in perfusion >40 minutes) at different doses (1, 3 and 10 mg/kg) and at different times after stroke onset (from 20 minutes to 4 hours). Control mice receive saline under identical conditions.

Primary outcome: lesion volume measured 24 h after stroke onset by thionine staining of one section in 10 (20 µm thick) covering the entire lesion.

Secondary outcomes: neuronal degeneration, blood brain barrier damage and motor impairment (behavioural tests; pole, coat hanger, open field, etc.)

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- ✓ **Comorbidities:** We have performed this experimental model of stroke in several strains of mice (C57Bl6J, TAFI and Mmp10 deficient mouse), with different comorbidities: aging, atherosclerosis (apolipoprotein E-deficient mouse) and streptozotocin-induced type 1 diabetes.