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Angiotensin-like 4 and ischaemic stroke: a promising start

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This editorial refers to ‘Protective effects of angiotensin-like 4 on cerebrovascular and functional damages in ischaemic stroke,’ by C. Bouleti *et al.*, doi:10.1093/eurheartj/eh153

Stroke is a global cause of morbidity and mortality, ranking fourth among all causes of death.¹ Although considerable progress has been made in developing effective tools for acute stroke treatment, at present the only drug approved is recombinant tissue plasminogen activator (rt-PA); thus, new strategies for its effective prevention and treatment are essential. Following ischaemia/reperfusion, the blood–brain barrier (BBB) becomes more permeable and, in doing so, it promotes an increased infiltration of pro-inflammatory cells, resulting in the so-called ‘reperfusion injury’.² Given its key role in mediating ischaemia/reperfusion-related neuronal damage, the BBB is a central target for the development of novel therapeutical strategies.

Bouleti and colleagues have provided an elegant study reporting a new target improving stroke outcome in mice.³ Their study shows that angiotensin-like 4 (ANGPTL4) modulates endothelial permeability following ischaemia/reperfusion and thus represents a potential new therapeutical target for the treatment of stroke.

ANGPTL4, first discovered in 2000, was originally classified as an adipokine playing roles in lipid metabolism.⁴ Over the last decade, ANGPTL4 has been recognized to play additional roles in tumorigenesis, angiogenesis, and redox regulation.⁴ Bouleti and colleagues investigated the role of ANGPTL4 in stroke by using a transient focal cerebral ischaemia murine model, where the middle cerebral artery was occluded for 1 h, followed by 24 h of reperfusion. Indeed, they could show that recombinant human ANGPTL4 (rhANGPTL4) treatment prior to the ischaemic episode greatly reduced the stroke size and consequent neurological deficit. To provide additional supporting evidence for the protective effects of ANGPTL4, Bouleti *et al.* analysed stroke outcome in ANGPTL4 knockout mice undergoing ischaemic stroke. Moreover, to translate their findings into a more clinically relevant experimental set-up, the authors treated mice with rhANGPTL4 upon reperfusion, 1 h after the ischaemic event. Both in ANGPTL4 knockout mice and in the post-ischaemic rhANGPTL4-treated mice, the authors could

confirm the protective effects of ANGPTL4 on stroke. Finally, to test the responsiveness of ANGPTL4 to stroke also in humans, Bouleti *et al.* stained brain samples of patients who had previously died from ischaemic stroke and demonstrated an increased expression of ANGPTL4.

Given the key role of vascular integrity in determining stroke outcome,² Bouleti and colleagues focused on analysing endothelial networks to characterize the protective effects of rhANGPTL4 treatment on stroke outcome. They demonstrated an improved endothelial network after ischaemia/reperfusion brain injury in the rhANGPTL4-treated mice as compared with controls, indicating a preservation of the vasculature during stroke. Cerebral endothelial cells build the initial barrier of the BBB separating blood components from brain cells, and are connected by tight and adherens junction proteins.⁵ A disruption of these cell–cell contacts leads to vascular leakage, oedema formation, and brain damage. In the present work, the integrity of tight and adherens junctions was assessed by characterizing claudin-5 and VE-cadherin, two main junction proteins.⁵ The authors showed an increase in claudin-5- and VE-cadherin-positive areas after ischaemia/reperfusion injury in the rhANGPTL4-treated mice compared with the control mice, indicating an effect of ANGPTL4 on preserving the integrity of tight and adherens junctions in stroke.

Acutely, vascular endothelial growth factor (VEGF) is a potent mediator of vascular permeability, and its antagonism has been shown to reduce ischaemia/reperfusion-related brain oedema and injury.⁶ Given the important role of VEGF as a mediator of acute vascular leakage following ischaemia⁷ (Figure 1), Bouleti *et al.* tested the possibility that rhANGPTL4 treatment may act by disrupting VEGF receptor 2 (VEGFR2) signalling. Indeed, they showed that ANGPTL4 maintains vascular integrity via inhibition of VEGF-induced, VEGFR2-mediated, disruption of VE-cadherin and claudin-5; a process in turn depending on Src phosphorylation and phosphoinositid-3 kinase (PI3K)/Akt activation (Figure 1).

Transmigration of circulating cells from the vasculature into the brain is a key step in tissue damage following ischaemia/reperfusion injury. This event is promoted, in part, by an up-regulation of

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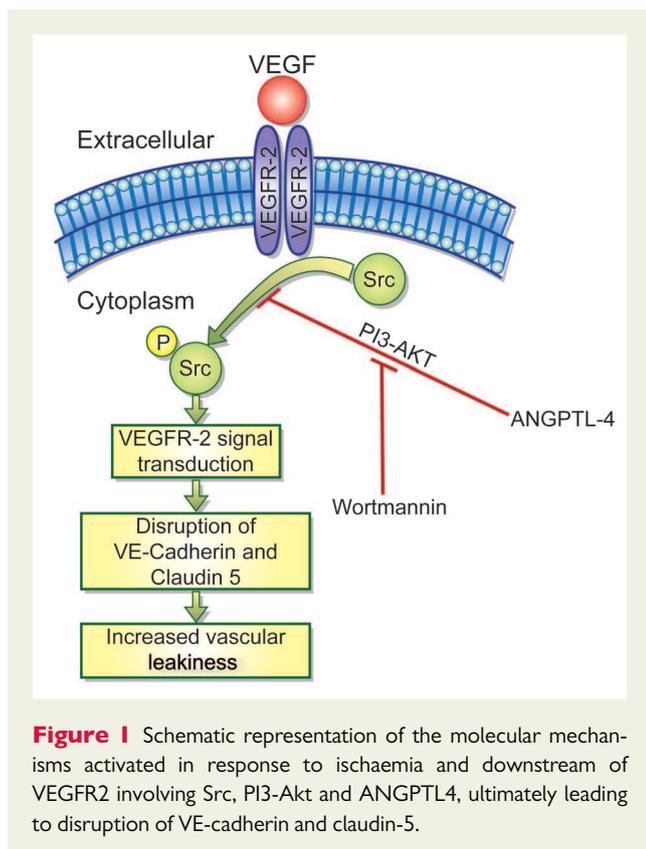


Figure 1 Schematic representation of the molecular mechanisms activated in response to ischaemia and downstream of VEGFR2 involving Src, PI3-Akt and ANGPTL4, ultimately leading to disruption of VE-cadherin and claudin-5.

endothelial adhesion molecules. Bouleti and colleagues demonstrated that rhANGPTL4 treatment reduces intercellular adhesion molecule-1 (ICAM-1) expression in the ipsilateral hemisphere of rhANGPTL4-treated mice as compared with controls, indicating that ANGPTL4 may also interfere with inflammatory responses following ischaemia/reperfusion.

In the clinical setting, rt-PA was first approved by the Food and Drug Administration (FDA) in 2006 for the treatment of ischaemic stroke within 3 h from symptom onset, after the publication of the NINDS trial,⁸ in which a 12% absolute (32% relative) increase in the number of patients with minimal or no disability was achieved in the rt-PA group compared with placebo. However, access to rt-PA is limited by numerous contraindications, which make this treatment available only for a minority of patients. In addition, rt-PA treatment is burdened by a major complication, i.e. haemorrhagic evolution of the ischaemic lesion, which is one of the principal reasons given by many emergency room physicians and neurologists for their avoidance of use of rt-PA therapy.

In their study, Bouleti *et al.* demonstrate that administration of ANGPTL4 in an ischaemic stroke model decreases infarct size and improves neurological function by maintaining vascular integrity through prevention of BBB breakdown.

From a clinical perspective, these data are very interesting, as we do know that BBB breakdown is involved in both ischaemic and haemorrhagic stroke, as well as in the haemorrhagic transformation of ischaemic stroke, either spontaneously or after rt-PA administration.

However, even if numerous neuroprotective agents have already been shown to be effective in ameliorating outcome in experimental models of stroke, to date clinical neurologists are faced with a long list

of failures in the translation into the clinical setting. To address this issue, the Stroke Therapy Academic Industry Roundtable (STAIR) published in 1999 recommendations to improve the quality of pre-clinical studies of acute stroke therapies,⁹ further updated in 2009.¹⁰ These guidelines include, first of all, methodological standards, such as random allocation, allocation concealment, sample size calculation, inclusion and exclusion criteria, randomization, reporting of animals excluded from analysis, and blinded assessment of outcome, as well as reporting of potential conflicts of interest and study funding. In addition, the authors underline the intrinsic limitations of animal stroke models, the most relevant being that ischaemia is experimentally induced in otherwise healthy animals, whereas human stroke occurs in the context of different risks factors and concomitant medications. Finally, accumulating data suggest that many of the neuroprotective targets tested in preclinical models may have a negative effect on the recovery process. Thus, any acute therapy must consider whether the desired target plays a role in the subsequent process of recovery. Accordingly, multiple endpoints, such as histological endpoints, biological markers, imaging data, and behavioural and functional outcomes, should be carefully identified and tested at the appropriate time points. In line with these considerations, many of the animal models demonstrated optimal benefit in a time frame that was not practical for clinical stroke intervention, such as either pre-treatment or within 90 min of the infarction. Because the onset-to-needle time in the real world is relatively long, the time window in animal experiments should be defined accordingly.

Bouleti and colleagues show very convincingly and in detail the vasculoprotective properties of ANGPTL4 in ischaemic stroke. In particular, the post-ischaemic treatment with rhANGPTL4 provides clinical relevance and sets the basis for follow-up investigations. However, there are also some limitations to this work, which should be mentioned. First of all, all analyses concerning stroke size, neurological deficit, and mechanisms are performed at short time points and no data are provided to confirm the protective effects of rhANGPTL4 treatment beyond 24 h. Given the known biphasic nature of some of the molecular signals activated during stroke, the role of ANGPTL4 in vascular integrity and stroke outcome should also be studied long term to elucidate fully its therapeutic potential. Secondly, most *in vitro* experiments are conducted on human endothelial cells of venous or dermal origin while we feel that mechanistic studies should also be confirmed on human endothelial cells originating from cerebral arteries to exclude possible cell type-specific effects.

Recently, stroke research reached beyond the role of the BBB; indeed, a number of other approaches have also been considered. Free radicals are important mediators of BBB damage following ischaemia; thus, different strategies to prevent the surge of reactive oxygen species have been successfully studied.^{11,12} Additionally, much focus has also been put on studying the therapeutic potential of stem cell transplantation in stroke patients. Although its effectiveness in stroke animal models¹³ as well as its clinical safety and feasibility were demonstrated,¹⁴ the clinical efficacy of stem cell therapy still has to be improved¹⁵ so as to become a concrete therapeutic alternative, and at present this scenario still seems far away.

We do hope that, if the results obtained by Bouleti and colleagues are confirmed by further experiments adhering to the suggested recommendations, any clinical trial implemented will not run into

previously observed trivialities. The world outside desperately needs effective new therapies to combat stroke, which every year causes millions of people to become disabled, and is likely to cause even more with the ageing population.

Conflict of interest: none declared.

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