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European Heart Journal Editorial

Angiopoietin-Like 4 and Ischemic Stroke: a Promising Start

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This editorial refers to 'Protective effects of Angiopoietin-Like 4 on cerebrovascular and functional damages in ischemic stroke', by Claire Bouleti et al., manuscript number D-12-02901R2

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 ischemia/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 ischemia/reperfusion-related neuronal damage, the BBB is a central target for the development of novel therapeutical strategies.

Bouleti and colleagues provided an elegant study reporting a new target improving stroke outcome in mice. Their study shows that Angiopoietin-like 4 (ANGPTL4) modulates endothelial permeability following ischemia/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[3]. Over the last decade, ANGPTL4 was recognized to play additional roles as also in tumorigenesis, angiogenesis and redox regulation[3]. Bouleti and colleagues investigated the role of ANGPTL4 in stroke by using a transient focal cerebral ischemia murine model, where the middle cerebral artery was occluded for 1 hour, followed by 24 hours of reperfusion. Indeed, they could show that recombinant human ANGPTL4 (rhANGPTL4) treatment prior to the ischemic episode strongly reduced stroke size and consequent neurological deficit. To provide additional supporting evidence for the protective effects of ANGPTL4, Bouleti et al. analyzed stroke outcome in ANGPTL4 knockout mice undergoing ischemic stroke. Moreover, to translate their findings into a more clinically relevant experimental setup, the authors treated mice with rhANGPTL4 upon reperfusion, 1 hour after the ischemic event. Both in ANGPTL4 knockout mice as well as in the post-ischemic 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 ischemic 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 analyzing endothelial networks to characterize the protective effects of rhANGPTL4 treatment on stroke outcome. They demonstrated an improved endothelial network after ischemia/reperfusion brain injury in the rhANGPTL4-treated mice as compared to controls, indicating a preservation of the vasculature during stroke. Cerebral endothelial cells build the initial barrier of the blood-brain barrier (BBB) separating blood components from brain cells, and are connected by tight and adherens junction proteins[4]. A disruption of these cell-cell contacts leads to

vascular leakage, oedema formation and brain damage. In the present work, integrity of tight and adherens junctions was assessed by characterizing Claudin-5 and VE-cadherin, two main junction proteins[4]. The authors showed an increase in Claudin-5 and VE-cadherin-positive areas after ischemia/reperfusion injury in the rhANGPTL4-treated mice compared to 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 ischemia/reperfusion related brain oedema and injury[5]. Given the important role of VEGF as a mediator of acute vascular leakage following ischemia[6] (Fig.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 (Fig.1).

Transmigration of circulating cells from the vasculature into the brain is a key step in tissue damage following ischemia/reperfusion injury. This event is promoted, in part, by an up regulation of 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 to controls, indicating that ANGPTL4 may also interfere with inflammatory responses following ischemia/reperfusion.

In the clinical setting, rt-PA was first approved by FDA in 2006 for the treatment of ischemic stroke within three hours from symptom onset, after the publications of NINDS trial[7], in which a 12 percent absolute (32 percent relative) increase in the number of patients with minimal or no disability was achieved in the rt-PA group compared to 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, ie. hemorrhagic evolution of the ischemic lesion, which is one of principal reasons given by many emergency room physicians and neurologists for their avoidance of use of rt-PA therapy.

The Authors demonstrate that administration of ANGPTL4 in an ischemic 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 both in ischemic and hemorrhagic stroke, as well as in the hemorrhagic transformation of ischemic stroke, either spontaneously or after rt-PA administration.

However, even if numerous neuroprotective agents have been already shown to be effective in ameliorating outcome in experimental models of stroke, up to now, clinical neurologists are faced with a long list of failures in the translation to the clinical setting. To address this issue, the Stroke Therapy Academic Industry Roundtable (STAIR) published in 1999 the recommendations to

improve the quality of preclinical studies of acute stroke therapies[8], further updated in 2009[9]. 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 ischemia 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 if the desired target plays a role in the subsequent process of recovery. Accordingly, multiple end-points, such as histological end-points, biological markers, imaging data, behavioral and functional outcomes, should be carefully identified and tested at the appropriate time-points. In line with these consideration, many of the animal models demonstrated optimal benefit at a timeframe that was not practical for clinical stroke intervention such as either pre-treatment or within 90 minutes 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 ischemic stroke. In particular, the post-ischemic 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 analysis concerning stroke size, neurological deficit, as well as mechanisms are performed at short time points and no data are provided to confirm the protective effects of rhANGPTL4 treatment beyond 24 hours. Given the known biphasic nature of some of the molecular signals activated during stroke, the role of ANGPTL4 on vascular integrity and stroke outcome should also be studied long-term to fully elucidate its therapeutical 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 ischemia thus different strategies to prevent the surge of ROS have been successfully studied[10, 11]. Additionally, much focus has also been put on studying the therapeutical potential of stem cell transplantation in stroke patients. Although its effectiveness in stroke animal models[12] as well as its clinical safety and feasibility were demonstrated[13], the clinical efficacy of stem cell therapy still has to be improved[14] so as to become a concrete therapeutical alternative and at present this scenario seems still far.

We do hope that, if the results obtained by Bouleti and colleagues will be 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 against stroke, which causes every year millions of disabled, and is likely to cause even more with the aging population.

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