Toward a Biological Therapy to Improve Stroke Outcomes After Thrombolytic Therapy

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Stroke is a devastating event, especially in the lives of the elderly, and remains a worldwide public health problem. In the United States alone, the prevalence of stroke is 8.8 million people, and stroke is responsible for 200,000 deaths annually, 1 of every 16 deaths.\(^1\) Every 40 s, someone in the United States has a new stroke, which results in 800,000 new strokes annually. Of those who do survive, many are disabled: stroke is the most common cause of disability worldwide, and costs associated with its effects are estimated to be \(>\$60\) billion per year in the United States alone.\(^2\)

More than 80% of strokes are attributable to ischemia that is caused by either thrombus or thromboembolism. Early in the 20th century, the development of antihypertensive drugs dramatically reduced the incidence of stroke. It was not until the end of the 20th century that a treatment was developed to improve outcomes after a stroke. Intravenous tissue plasminogen activator administered within 4.5 hours after stroke onset is the only proven therapy for improving outcomes in patients with acute ischemic stroke.\(^3\) For large-artery strokes unresponsive to thrombolytic therapy, endovascular recanalization therapy achieves a higher recanalization rate and, in so doing, reduces disability and death according to a recent meta-analysis.\(^3\)

The rapid reperfusion after thrombolytic therapy also has deleterious effects. Ischemia-reperfusion causes increased oxidative stress and inflammation in the border zone that increases cell death and limits functional recovery. Over the past few decades, substantial work has been undertaken to develop treatments to reduce the ischemia-reperfusion injury of thrombolytic therapy and thereby reduce further the disability and death after acute ischemic cerebral infarctions. It is in this context that the work of Dhanesha et al\(^4\) raises hope that a biological therapy can be developed to reduce the clinical consequences of ischemia-reperfusion injury after thrombolytic therapy for acute stroke.

By providing substrate for the adhesion and spreading of cells, fibronectin (Fn), an adhesive glycoprotein, plays critical roles in tissue response to inflammation and thrombosis.\(^5\) By alternative splicing of the primary transcript, Fn has 2 isoforms, extra domain A (Fn-EDA) and extra domain B. In the plasma under normal conditions, Fn lacks both domains, whereas cellular Fn contains either domain A or B.\(^5\)

In their study in this issue of Circulation, Dhanesha et al\(^4\) used multiple single knockouts and double knockouts to characterize the role of Fn-EDA in the ischemia-reperfusion injury in a hypercholesterolemic mouse model of transient severe cerebral ischemia. They show conclusively in a compelling preclinical model that Fn-EDA plays a central role in the pathogenesis of cerebral ischemia-reperfusion injury and neurological outcomes.

Consistent with the human condition, healthy mice have negligible concentrations of Fn-EDA in their plasma. However, the concentration of Fn-EDA increases significantly in mice that are hypercholesterolemic, analogous to the increased concentrations of Fn-EDA in humans who have atherosclerosis. In Fn-EDA–/– ApoE–/– double knockout mice, after 60 minutes of total middle cerebral artery occlusion, followed by 23 hours of reperfusion, the volume of cerebral infarction and the neurological outcomes were substantially improved over ApoE–/– mice. To determine the durability of the salutary effect, they studied the mice for 8 additional days. The Fn-EDA–/– ApoE–/– mice had a 60% higher survival rate, and improved neurological outcomes, as well, at both days 3 and 5 after induction of cerebral ischemia. And in an effort to strengthen their preclinical model by following the updated Stroke Treatment Academic Industry Roundtable recommendations, they show that sex has no effect on neurological outcome.

To determine whether the improved neurological outcome in the Fn-EDA–/– ApoE–/– mice was because of increased blood flow, they used laser Doppler to show that, in fact, the local cerebral blood flow was increased in these mice. They also show that intracerebral fibrinogen content was lower in Fn-EDA–/– ApoE–/– mice, suggesting less arterial thrombosis. To test their hypothesis, they used a carotid artery thrombosis model that measured the growth kinetics of the thrombus and the time to carotid artery occlusion and found that both variables were significantly reduced in Fn-EDA–/– ApoE–/– mice.

To identify the molecular mechanism by which Fn-EDA impairs neurological recovery after cerebral ischemia-reperfusion, the authors measured neutrophil and macrophage influx within the infarct and peri-infarct regions of the perfused brain and found a marked reduction in these 2 cell types in the Fn-EDA–/– ApoE–/– mice. They then measured toll-like receptor 4 (TLR4) expression and phospho-IKK\(\alpha/\beta\) and phospho-NF\(\kappa\)B p65, which are downstream components of the canonical signaling pathway in the infarcted...
and surrounding ischemic border zone areas following total middle cerebral artery occlusion. Immunoblots showed a \( \approx 2 \)-fold reduction in phospho-IKK\( \alpha/\beta \) and phospho-NFkB p65 intensity levels in Fn-EDA\(^{-/-}\) ApoE\(^{-/-}\) mice. Consistent with this finding, enzyme-linked immunosorbent assay results also showed a 2-fold reduction in phospho-NFkB p65, tumor necrosis factor-\( \alpha \), and interleukin-1\( \beta \). Finally, to characterize the specificity of this finding, they showed that the infarct volume and the neurological outcome of Fn-EDA–/– ApoE–/– mice were similar to the results observed in the Fn-EDA–/– ApoE–/– TLR4\(^{-/-}\) triple knockout mice.

To determine the cell type that generates the Fn-EDA, they created chimeric mouse models by transplanting bone marrow from Fn-EDA–/– ApoE–/– mice or ApoE–/– mice into lethally irradiated Fn-EDA–/– ApoE–/– mice. In the latter chimera, Fn-EDA is only expressed in cells of hematopoietic origin. The results of these studies showed that the source of Fn-EDA is not from cells of hematopoietic stem cell origin and most likely from endothelial cells.

To determine if a biological therapeutic could be developed based on the identification of Fn-EDA as a primary mediator of the pathological effect of hypercholesterolemia on recovery from cerebral ischemia-reperfusion injury, a blocking anti-Ig antibody to Fn-EDA was developed. When administered 15 minutes after reperfusion, regional blood flow was increased that reduced infarct volumes 2-fold and improved neurological outcomes, as well. The inflammatory cytokines phospho-NFkB p65, tumor necrosis factor-\( \alpha \), and interleukin-1\( \beta \) were concomitantly reduced significantly.

Although preliminary, the effectiveness of the anti-Ig antibody to Fn-EDA raises hope for its potential to develop into a biological therapeutic to improve neurological outcomes after thrombolytic therapy for acute ischemic stroke. It might also lengthen the duration of ischemia for which thrombolytic therapy may be given safely to people who have an acute ischemic stroke. Sometimes, the lack of translation of findings in mouse models to the human condition is largely attributable to the specific conditions of a preclinical model. In this case, the use of a hypercholesterolemic mouse that showed identical patterns of expression of Fn-EDA in humans increased the likelihood of translating these findings to a human trial. It remains to be established if other cardiovascular risk factors such as type 2 diabetes mellitus have the same effect on the plasma concentration of Fn-EDA.

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None.

**References**

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