Shooting vascular oxidative stress: new hopes for stroke patients?

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This editorial refers to ‘Post-ischaemic silencing of p66Shc reduces ischaemia/reperfusion brain injury and its expression correlates to clinical outcome in stroke’, by R.D. Spescha et al., on page 1590.

Stroke is the second leading cause of disability after ischaemic heart disease.1,2 The World Health Organization estimates that the number of stroke events in Europe is projected to rise from 1.1 million in 2000 to 1.5 million per year by 2025.3 In spite of the significant advances in clinical management and care, over half of patients aged ≥45 years (52% of men and 56% of women) will die within 5 years of a stroke.1 About 60% of stroke survivors will recover functional independence within 3 months of onset whereas 20% will require institutional care.1 Alarming, stroke-related morbidity implies tremendous costs, with an annual European expenditure of €64.1 billion in 2010 alone,4 as well as a heavy burden for individuals, families, and society.

Breakthrough therapies have yet to be approved and there are still no highly effective acute treatments available. Over the past 20 years, clinical research has focused on the development of reperfusion therapies for acute ischaemic stroke, including systemic i.v. thrombolytics as well as endovascular reperfusion therapies including intra-arterial thrombolysis, mechanical thrombectomy, and thromboaspiration.5 I.v. thrombolysis with tissue plasminogen activator (tPA) remains the only evidence-based treatment for acute ischaemic stroke.5 About 60% of stroke survivors will recover functional independence within 3 months of onset whereas 20% will require institutional care.1 Alarming, stroke-related morbidity implies tremendous costs, with an annual European expenditure of €64.1 billion in 2010 alone,4 as well as a heavy burden for individuals, families, and society.

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The realization that post-ischaemic p66Shc phosphorylation is emerging as a key transduction pathway relevant to oxidative stress and endothelial damage.10 This protein functions as a redox enzyme implicated in mitochondrial ROS generation and translation of oxidative signals into apoptosis.10 Several chronic stimuli trigger p66Shc phosphorylation, allowing transfer of the protein from the cytosol to the mitochondrion where it catalyses ROS production via cytochrome c oxidation.11 Spescha et al. have now demonstrated that targeting the adaptor p66Shc prevents ischaemia/reperfusion (I/R) brain injury in mice, and provide translational insights on the relationship between p66Shc expression and short-term outcome in stroke patients.12 The authors show that post-ischaemic in vivo silencing of p66Shc preserved BBB integrity, resulting in smaller lesion volumes, decreased neurological deficits, and improved survival of mice.12 Mechanistic experiments in brain microvascular endothelial cells revealed that hypoxia/reoxygenation elicited p66Shc phosphorylation at Ser36, thus leading to increased
generation of superoxide anion and reduced availability of nitric oxide (NO), a major guarantor of vascular health. Interestingly, p66Shc-dependent ROS generation was responsible for activation of NADPH oxidase, thus amplifying endothelial generation of free radicals. Indeed, knockdown of p66Shc blunted NADPH activity and ROS formation while restoring NO levels. Furthermore, this work unveils a new molecular connection between p66Shc and claudin-5, a key tight junction protein serving as a physical barrier to prevent passage of solutes and water through the paracellular space. In vitro experiments showed that hypoxia/reoxygenation blunted claudin-5 levels whereas p66Shc down-regulation or use of antioxidants restored its expression, suggesting that ROS critically impair endothelial integrity by dampening claudin-5 (Figure 1). Whether p66Shc directly modulates claudin-5 function, regardless of ROS, remains elusive and requires further molecular investigation.

In order to translate their experimental data to the human setting, p66Shc gene expression levels were assessed in peripheral blood mononuclear cells isolated from 27 patients with ischaemic stroke and age-matched healthy controls. In line with the in vivo and in vitro results, p66Shc expression was significantly increased in stroke patients and positively correlated with neurological deficits at admission, measured according to the National Institutes of Health Stroke Scale (NIHSS). An interesting observation from this study was that p66Shc expression in stroke patients correlated with short-term neurological outcome only in subjects receiving thrombolytic therapy, suggesting that p66Shc is mostly acting during the reperfusion phase. In other words, activation of p66Shc following vessel recanalization might propagate cerebral ischaemic damage by triggering BBB disruption in a ROS-dependent manner. The present study provides evidence that a mechanism-based approach, namely delivery of small interfering RNA (siRNA) against p66Shc, can be implemented to prevent post-ischaemic cerebral damage. The possibility of translating this approach to stroke patients is not unrealistic since RNA interference (RNAi)-based strategies are being tested in several clinical feasibility studies. Current challenges concerning their use entail target/sequence validation, tissue specificity, transfection efficiency, and mitigation of unwanted off-target effects.

In contrast to the present findings, Brown et al. have previously shown that p66Shc may confer neuronal protection in an experimental model of brain ischaemic pre-conditioning. In vitro studies in neurons showed that p66Shc can be actively recruited to stressed mitochondria where it promotes energetic compensation by inducing the expression of heat shock protein 70 (HSP70), an important gatekeeper against cellular stress. However, in a separate investigation published in 2013, Spescha et al. have clearly demonstrated that mice with genetic deletion of p66Shc (p66Shc−/−) are protected against I/R brain injury. Indeed, p66Shc−/− mice displayed a significantly reduced stroke size, preserved neurological function, and decreased production of free radicals. The validity of these findings is supported by the employment of a genetic approach which has the unique advantage of providing reliable read-outs on the role of p66Shc during I/R injury. They also found that reperfusion injury in wild-type mice induced p66Shc up-regulation in the basilar and middle cerebral artery, but not in brain tissue, suggesting a predominant
involvement of vascular p66Shc.14 In their current study,15 Spescha et al. further show that reduced stroke size is mostly driven by endothelial p66Shc down-regulation after in vivo RNAi. The use of a dye-labelled p66Shc siRNA demonstrated that 21.2% of brain endothelial cells were positive whereas siRNA was detectable only in 0.6% of leukocytes and 0.2% of other nucleated cells. These findings are in line with the earlier demonstration that in vivo delivery of p66Shc siRNA mostly distributes to the vascular endothelium while sparing other cell types.15 Taken together, the findings reported so far suggest that activation of p66Shc in the brain vasculature is likely to be identified as a key driver of post-ischaemic cerebral damage rather than a compensatory and perhaps futile mechanism counteracting neuronal damage (Figure 1).

Another interesting implication of the present work is that p66Shc expression might serve as a potential biomarker to monitor I/R injury in humans.15 Data collected in peripheral blood monocytes isolated from stroke patients showed that p66Shc expression levels were transiently increased 6 h after initial stroke symptoms and returned to control levels after 24 h. Based on these results, one can postulate that p66Shc up-regulation may be useful in identifying patients with ongoing cerebral ischaemia, especially when clinical presentation remains obscure due to the lack of specific symptoms. Further studies are needed to understand the significance of p66Shc up-regulation in peripheral monocytes and its reliability as a potential biomarker in this area. Undoubtedly, the work by Spescha et al. is a good example of translational research linking basic findings to the clinical setting. The present results contribute to building a solid ground for a ‘lab to market’ approach addressing whether p66Shc can be considered as a novel molecular target and/or a diagnostic tool in patients with ischaemic stroke.

Conflict of interest: none declared.

References