EDITORIAL

Molecular mechanisms underlying neurovascular protection in stroke

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This special Neuroscience issue of *The Journal of Physiology* includes five review articles based on *The Journal*'s sponsored symposium *Molecular mechanisms underlying neurovascular protection in stroke* held at Experimental Biology in Washington, DC, USA on 9–12 April 2011. These invited review articles provide an up-to-date overview of basic and clinical research focused on characterizing the cellular mechanisms underlying protection of the neurovascular unit in stroke. Understanding the molecular mechanisms underlying injury and repair processes in the brain following stroke will provide valuable insights for the development of drugs and/or natural plant-derived compounds to protect the brain from ischaemia–reperfusion injury.

Stroke is associated with one of the highest rates of mortality, with ∼150,000 cases each year in the UK. The failure of bench-to-bedside translation highlights the importance of understanding the mechanisms underlying damage to the brain and the processes involved in its repair. Tissue plasminogen activator (tPA) is the only approved treatment for stroke, but its beneficial thrombolytic actions are counterbalanced by the limited window of efficacy following the onset of symptoms and its neurotoxicity. Ischaemia–reperfusion injury after stroke leads to disruption of the blood–brain barrier causing potentially fatal cerebral oedema and an increased incidence of haemorrhage. The initial rapid loss of viable brain tissue in the ischaemic core region is often followed by subsequent damage to the surrounding penumbra. Rescue within the penumbra is a key objective for stroke research, and targeting endogenous defence mechanisms by which the brain protects itself and recovers from ischaemic damage should provide novel insights for effective treatment strategies. Although ischaemic preconditioning ('tolerance to ischaemia') has been employed to improve endogenous antioxidant defences in the brain, the cellular mechanisms underlying protection of the neurovascular unit remain to be elucidated. This series of timely reviews highlights important advances in the field and should be of interest to both clinical and basic researchers interested in therapeutic strategies for the treatment of stroke and ageing-related neurodegenerative diseases.

Studies in experimental rodent models of stroke suggest that neuroprotective agents targeted to the brain parenchyma may reduce ischaemic injury via associated changes in cerebral blood flow, but these findings have failed to translate into the clinic. Alastair Buchan and colleagues (Sutherland *et al.* 2011) critically review the evidence that an improvement in cerebral blood flow is important, but may not be necessary, for protection of the brain against ischaemia–reperfusion injury in stroke. Neuroprotective treatments evoking multiple cellular responses such as activation of hypoxia inducible factor (HIF) and its downstream targets endothelial nitric oxide synthase, VEGF and erythropoietin complemented by augmented cerebral blood flow may ultimately ensure translation into clinical benefit.

As reviewed by Bernhard Nieswandt and colleagues, the cellular interactions leading (Nieswandt *et al.* 2011) from thromboembolic vessel occlusion to development of an infarct in the brain parenchyma in stroke remain poorly understood. These authors provide an overview of the basic mechanisms of thrombus formation and the use of anti-thrombotic treatment in clinical stroke. Recent evidence suggests that thrombus formation and immune-mediated processes are closely correlated in the pathogenesis of cerebral ischaemia, contributing significantly to brain damage in stroke. Early platelet adhesion/activation, involving von Willebrand factor (vWF) receptor glycoprotein Ib, its ligand vWF and the collagen receptor GPVI, plays a key role in the development of an infarct following transient ischaemia–reperfusion. Interestingly, as highlighted by these authors, immunodeficient mice lacking T-cells are protected markedly against focal brain ischaemia.

Activation of the redox sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) has been reported to protect the brain against reactive oxygen species (ROS)-mediated injury following ischaemia–reperfusion injury in stroke. Giovanni Mann and colleagues (Alfieri *et al.* 2011) review the evidence that activation of the Nrf2-Keap1 antioxidant defence pathway may serve as a potential therapeutic target for neurovascular protection. Downstream targets of Nrf2 such as inducible haem oxygenase-1 (HO-1) appear to play a key role in protecting the blood–brain barrier, astrocytes and neurons against oxidative stress induced damage. In this context, Nrf2 and HO-1 deficient mice subjected to transient middle cerebral artery occlusion (MCAO) exhibit significantly greater brain infarcts following reperfusion. The authors summarise the recent literature that certain drugs and natural plant-derived compounds can activate the Nrf2–Keap1 defence pathway to protect the brain against stroke. As ageing is associated with an increased incidence of stroke, and Nrf2 expression and activity diminishes with ageing, it will be important to examine the molecular mechanisms underlying Nrf2 mediated redox signalling in young and aged rodent models of stroke. A better understanding of the actions of new drugs and natural compounds as inducers of Nrf2 may provide valuable insights for therapeutic interventions to protect the neurovascular unit not only in rodent models but also in humans.

As reviewed by Constantino Iadecola and colleagues (2011), short periods of non-lethal cerebral ischaemia can reduce damage to the brain induced by a subsequent major ischaemic insult. In the context of the brain 'ischaemic tolerance' can develop within minutes or hours after the 'preconditioning' stimulus (early preconditioning) or occur after days of the inducing stimulus and last for weeks (delayed preconditioning). These authors examine the role of nitric oxide (NO) in early and delayed preconditioning, highlighting that NO is protective in the early phase of ischaemia when cerebral

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blood flow may limit tissue damage but destructive in the late phase when increased NO generation leads to mitochondrial dysfunction and DNA damage. In terms of a clinical application, targeting NO to specific brain tissue and cells remains a challenge for future research initiatives.

Ulrich Dirnagl and Phillip Mergenthaler (Mergenthaler & Dirnagl, 2011) provide a valuable insight into the use of pre-, per-, post- and remote 'conditioning' as tools to unravel the molecular mechanisms underlying endogenous neuroprotection in stroke. As described by the authors, preconditioning involves a conditioning stimulus (e.g. hypoxia, metabolic inhibition or subthreshold stimulus) given days or minutes before ischaemia; perconditioning involves giving the conditioning stimulus while a noxious stimulus is still present; postconditioning involves giving a conditioning stimulus after reperfusion; and remote conditioning involves for example limb ischaemia resulting in protection of the brain. In view of the complexity of these different 'conditioning' strategies to protect the brain against stroke, these authors emphasise the importance of conducting preclinical research of the highest quality as a basis for future clinical trials.

In summary, the molecular mechanisms underlying neurovascular protection afforded by specific drugs and natural compounds requires further study, in particular the upregulation of endogenous antioxidant defence genes in different brain cell types in stroke. Our studies of the beneficial actions of soy isoflavones on endothelial NO production, vascular reactivity, and arterial blood pressure *in vivo* have identified the Nrf2–Keap1 pathway as an important therapeutic target to combat oxidative stress in vascular disease (Gao & Mann, 2009; Siow & Mann, 2010; Cheng *et al.* 2011; Rowlands *et al.* 2011). Given the challenges facing treatment strategies for stroke, further preclinical research on the mechanisms regulating endogenous defence pathways in stroke is warranted. Unravelling the neurogenic, immunological, genetic and epigenetic influences underlying responses of different brain cell types to ischaemia–reperfusion injury should provide novel insights for clinical translation.

References

Alfieri A, Srivastava S, Siow RC, Modo M, Fraser PA & Mann GE (2011). Targeting the Nrf2–Keap1 antioxidant defence pathway for neurovascular protection in stroke. *J Physiol* **589**, 4125–4136.

Cheng X, Siow RC & Mann GE (2011). Impaired redox signaling and antioxidant gene expression in endothelial cells in diabetes: a role for mitochondria and the nuclear factor-E2-related factor 2-Kelch-like ECH-associated protein 1 defense pathway. *Antioxid Redox Signal* **14**, 469–487.

Gao L & Mann GE (2009). Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling. *Cardiovasc Res* **82**, 9–20.

Iadecola C, Kahles T, Gallo EF & Anrather J (2011). Neurovascular protection by ischaemic tolerance: role of nitric oxide. *J Physiol* **589**, 4137–4146.

Mergenthaler P & Dirnagl U (2011). Protective conditioning of the brain: expressway or roadblock? *J Physiol* **589**, 4147–4156.

Nieswandt B, Kleinschnitz C & Stoll G (2011). Ischaemic stroke: a thrombo-inflammatory disease? *J Physiol* **589**, 4115–4124.

Rowlands DJ, Chapple S, Siow RC & Mann GE (2011). Equol-stimulated mitochondrial reactive oxygen species activate endothelial nitric oxide synthase and redox signaling in endothelial cells: roles for F-actin and GPR30. *Hypertension* **57**, 833–840.

Siow RC & Mann GE (2010). Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis? *Mol Aspects Med* **31**, 468–477.

Sutherland BA, Papadakis M, Chen RL & Buchan AM (2011). Cerebral blood flow alteration in neuroprotection following cerebral ischaemia. *J Physiol* **589**, 4105–4114.

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