British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01564.x
www.brjpharmacol.org

COMMENTARY

Novel insights into the delayed vasospasm following subarachnoid haemorrhage: importance of proteinase signalling

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Keywords
cardiovascular pharmacology; drug receptor mechanisms; molecular pharmacology; anticoagulant/thrombolytic drugs; coagulation/fibrinolysis; control of smooth muscle; inflammation; antioxidants/NO pathways drugs; GPCRs; endothelin

Received
19 June 2011
Accepted
21 June 2011

This article summarizes the findings of Kameda *et al.* (this issue of BJP) that suggest a new avenue for the pharmacological treatment of subarachnoid haemorrhage (SAH) involving the combined use of a proteinase inhibitor (argatroban) that targets thrombin and an antioxidant (vitamin C). The findings are presented in the context of previous modalities of treating SAH that are of modest impact and the possibility that inhibiting proteinase-mediated signalling via proteinase-activated receptors like the thrombin PAR1 receptor combined with blocking oxidative stress may provide a new avenue for the treatment of SAH.

LINKED ARTICLE
This article is a commentary on Kameda *et al.*, pp. 106–119 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01485.x

Overview

Spasm of the intracranial arteries following subarachnoid haemorrhage (SAH) was documented angiographically over 60 years ago (Ecker and Riemenschneider, 1951). The vascular response to the presence of blood in the subarachnoid space exhibits a puzzling delayed time frame (Weir *et al.*, 1978), with a substantial interval of 3 to 4 days before the onset of vasoconstriction that peaks 7 to 10 days after the initial bleed and resolves by day 18. In the current issue of the BJP, Kameda *et al.* (2011) highlight the importance of proteinases in the delayed vasospasm that may also involve proteinase-generated endothelin-1.

Mechanisms currently proposed for SAH-induced delayed vasospasm

As recently summarized (Kolias *et al.*, 2009; Pluta *et al.*, 2009), the insult of extravasated blood and associated breakdown products generates persistent vasospasm via both Ca^{2+}-dependent and Ca^{2+}-independent contractile processes. Proposed mechanisms (see Weir *et al.*, 1999) include (i) free radicals and oxidative stress generated by oxyhaemoglobin; (ii) sequestration of NO; (iii) generation of arachidonate metabolites; (iv) products of the complement cascade; (v) increased transcription of inflammatory mediators such as IL-1, IL-6 and IL-8; (vi) proteolytic generation of endothelin-1 by the endothelium and other CNS parenchymal cells like astrocytes and neurons; and (vii) proteinases, targeted by the serine proteinase inhibitor, FUT-175/Nafamastat.

Proteinase-mediated signalling, vasoregulation and neurodegeneration

Over the past 10 years, it has become evident that proteinases such as those of the coagulation cascade (e.g. thrombin, Factor VIIa/Xa) can signal to the vasculature not only by the generation of vasoactive peptides like endothelin-1 from polypeptide precursors but also by activating a novel four-member family of proteinase-activated GPCRs (so-called

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'PARs' 1 to 4; Hollenberg and Compton, 2002; Coughlin, 2005; Ramachandran and Hollenberg, 2008; Adams *et al.*, 2011).

PAR activation involves the proteolytic unmasking of a cryptic N-terminal sequence that acts as a 'tethered ligand' (TL) to activate the PAR receptor. Of relevance in SAH, both PARs 1 and 2 can regulate not only vascular tone but also neuronal function. Thus, thrombin, a key activator of PAR1, unmasks the 'tethered' ligand sequence, SFLLRN, that in turn activates vascular endothelial and smooth muscle PAR1 to cause either vasodilatation (endothelium) or vasoconstriction (smooth muscle). On balance, the constrictor action of PAR1 activation outweighs the vasodilator response and the vessel contracts. Synthetic peptides based on the TL sequence, like TFLLR-amide, can also selectively activate these PAR1 responses triggered by thrombin.

Since thrombin is one of the major 'blood products' activated at the site of SAH, Hirano and Hirano (2010) hypothesized that it would play a significant role via PAR1 activation. Using a rabbit SAH model (double administration of non heparin treated, autologous blood into the cisterna magna), they showed (i) that haemorrhage causes an up-regulation of the thrombin PAR1 receptor, with an accompanying increase in thrombin/PAR1-induced vascular contractility, and (ii) that in the same rabbit SAH model, a PAR1 antagonist, can diminish the increase in the thrombin contractile response that follows the instillation of blood into the subarachnoid space. Now, as reported in this issue of the BJP, Kameda *et al.* (2011) demonstrate changes in PAR1 pharmacodynamics (increased contractility; decreased desensitization) following exposure of the subarachnoid space to blood products. In particular, they used an antioxidant (vitamin C or tempol) in combination with a thrombin inhibitor (argatroban) to reverse these effects. This combined therapy not only attenuated the enhanced vascular contractile response induced by PAR1 activation but also diminished that for other G-protein-coupled contractile agonists such as vasopressin.

This exciting study thus points to a generalized dysfunction of GPCR sensitization–desensitization that is triggered for some agonists (thrombin, vasopressin) but not others (angiotensin II and prostaglandin $F_{2\alpha}$). An added, fascinating feature is the demonstration that combined thrombin inhibitor–antioxidant treatment is effective, *even if instituted two days after the first administration of blood into the cisterna magna*. That thrombin itself acting via its PAR1 receptor is involved in the post-SAH-induced changes is now validated by the use of both thrombin and PAR1 inhibitors. The rationale for the added effect of an antioxidant can be attributed not only to its impact on free radicals generated by oxyhaemoglobin but also to the potential block of NADPH-oxidase effects known to be triggered by PAR1 and other GPCRs.

Remaining questions

Is the delayed neurological deficit of SAH putatively caused by thrombin and other proteinases due only to vasospasm? This issue is highly relevant. Although the therapeutic use of an endothelin A receptor antagonist to mitigate post-SAH vasospasm is effective in diminishing the increased vasoconstriction (Vajkoczy *et al.*, 2005), it is disappointingly unsuccessful in improving mortality, morbidity or functional benefit in SAH patients (Macdonald *et al.*, 2011). It must be emphasized that proteinase signalling via the PARs affects not only the vasculature but also neuronal and astrocytic function, triggering neuroinflammatory and neurodegenerative processes (Noorbakhsh *et al.*, 2003). Thus, thrombin, via PAR1, can cause morphological changes in astrocytes and the release of inflammatory mediators as well as activating microglia with the potential to cause neuronal injury. Thrombin derived either from the extravasated blood or produced locally by CNS cells can contribute to this effect. Depending on its concentration, thrombin can have either neurodegenerative or neuroprotective effects presumably in part via PAR1.

Thus, targeting thrombin with an inhibitor like argatroban may have therapeutic implications beyond preventing thrombin's effects on the vasculature. Furthermore, other CNS-derived proteinases (like kallikrein-related peptidase-6; KLK6), generated locally in response to SAH, can in principle regulate PAR signalling on neuronal and other cells at the site of injury (Oikonomopoulou *et al.*, 2006). In particular, both PARs 1 and 2 that can be regulated by serine proteinases in addition to those of the coagulation pathway have been linked to neurodegenerative processes. In addition, activation of both of these GPCRs can trigger the production of reactive oxygen species via NADPH-oxidase. In summary, it can be said that the proteinases liberated by SAH including both those from the blood stream and those produced locally in the CNS in response to the trauma may target both the vasculature *and* neuronal cell elements accounting for the clinical and pathological features of SAH.

Looking to the future

To date, apart from endovascular intervention with angioplasty and hypervolemic haemodilution together with inotropic support, or 'triple H therapy', the pharmacological treatment of SAH has been surprisingly unrewarding. Some benefit from the use of calcium channel blockers and other vasodilators has been observed, but the effect of these agents may not be due to the mitigation of vasospasm but rather to their neuroprotective effects.

To sum up, the present study, which highlights the use of proteinase inhibitors along with an antioxidant, represents a potentially major advance. Significantly, the successful clinical use of a potent serine proteinase inhibitor, FUT-175/Nafamastat (Yanamoto *et al.*, 1992), to improve outcomes in SAH patients may be of relevance to the new findings reported here. Not only might FUT-175/nafamastat block thrombin activity, but it would also inhibit other serine proteinases that can signal via PAR activation. Proteinase inhibition could also block the generation of complement-derived and other vasoactive/neurotrophic peptides at the site of injury.

Given these considerations, the new study from the Hirano laboratory (Kameda *et al.*, 2011) along with previous complementary findings of others should stimulate further work to assess the potential roles of proteinases and their signalling mechanisms in causing the untoward sequelae of SAH.

Acknowledgements

The author is indebted to Dr Garnette Sutherland of the University of Calgary Faculty of Medicine, Division of Neurosurgery, Department of Clinical Neurosciences for his invaluable clinical perspective embedded in the text and to Dr Arthur Weston, whose editorial suggestions went 'above and beyond' to help crystallize the thoughts in this commentary. The input of Dr Francis E. LeBlanc is also much appreciated. Any possible errors of fact are entirely the author's responsibility. Work in the author's laboratory is supported largely by grants from the Canadian Institutes of Health Research and from the Heart & Stroke Foundation of Alberta, NWT and Nunavut.

Conflicts of interest

None.

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