Pharmacologically targeting inflammation and improving cerebrospinal fluid circulation improves outcome after subarachnoid haemorrhage

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Subarachnoid haemorrhage (SAH) is a feared type of haemorrhagic stroke characterised by high mortality (around 35%) and morbidity with only about 30% of patients being able to return to independent living. Acute or delayed cerebral ischemia resulting in further neurological deterioration and caused by severe arterial vasospasms in the macro- and microvasculature significantly contributes to poor SAH outcome. The exact mechanisms causing these vasospasms are not well understood, but it is believed that coagulation factors, haemoglobin breakdown products and in particular free iron from haemolytic erythrocytes are involved.[1] Blood breakdown products have also been identified as a potent inflammatory stimulus and can cause widespread neuronal damage and death.[2] Moreover, blood clots forming in the subarachnoid space are associated with disturbed cerebrospinal fluid (CSF) circulation leading to intracranial pressure increases, oedema formation, and hydrocephalus.[3] Timely surgical intervention to prevent re-bleeding is a key element of SAH treatment, however, few other causal treatments are available yet. Hence, additional treatment options supporting surgical interventions are urgently needed to improve outcome after SAH.

A recent study by Fang *et al.* in eBioMedicine describes a pharmacological approach to improve outcome after SAH using VX-765 (Belnacasan).[4] VX-765 is a prodrug that is converted by plasma esterases into VRT-043198, which potently inhibits caspase-1 (inhibitory constant (Ki) = 0.8 nM).[5] Caspase-1 (or interleukin (IL)-converting enzyme (ICE)) is a highly conserved enzyme that cleaves pro-IL-1β and pro-IL-18, activating these central inflammatory cytokines. Caspase-1 also cleaves gasdermin D, a mediator of pyroptosis, a form of regulated pro-inflammatory cell death.[6] Furthermore, the caspase-1-gasdermin-pyroptosis pathway has been demonstrated to be involved in the release of the procoagulant tissue factor (TF) from macrophages,[7] a protein which plays a central role in impaired CSF circulation after SAH.[3] Therefore, the authors hypothesised that blocking caspase-1 by VX-765 would induce strong anti-inflammatory and anti-coagulant effects and improve CSF circulation after SAH.

A major strength of the study by Fang *et al.* is the strong translational approach combining cell culture experiments with rodent studies and a small exploratory investigation in human SAH patients. CSF samples from 40 SAH patients and 4 healthy controls were investigated by mass spectrometry, expectedly showing a profound activation of both the intrinsic and extrinsic coagulation cascades. A significantly increased concentration of caspase-1 and TF in the CSF of 36 patients was also confirmed by ELISA. Higher TF concentrations were associated with a higher incidence of delayed cerebral ischaemia and hydrocephalus. This confirmed the relevance of the therapeutic target in human SAH patients.

This study also evaluated the time course of caspase-1 and TF expression in a rodent SAH model, revealing increased expression from 12 h after SAH, peaking at 72 h, and lasting up to 7 days in the case of TF. VX-765 was administered intranasally at 1, 25, and 49 h after SAH induction to dampen caspase-1 expression. VX-765

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treatment improved CSF circulation and blood clearance. This was confirmed in two different SAH models (perforation of the bifurcation of the anterior and the middle cerebral artery, autologous blood injection into the cisterna magna), a rarely seen feature in experimental studies, but strongly recommended for translational approaches in related diseases such as ischaemic stroke (please see[8] for review).

Caspase-1 inhibition further reduced the inflammatory response and fibrin deposition after SAH. As expected, it reduced the number of pyroptotic cells and abrogated inflammasome activation including

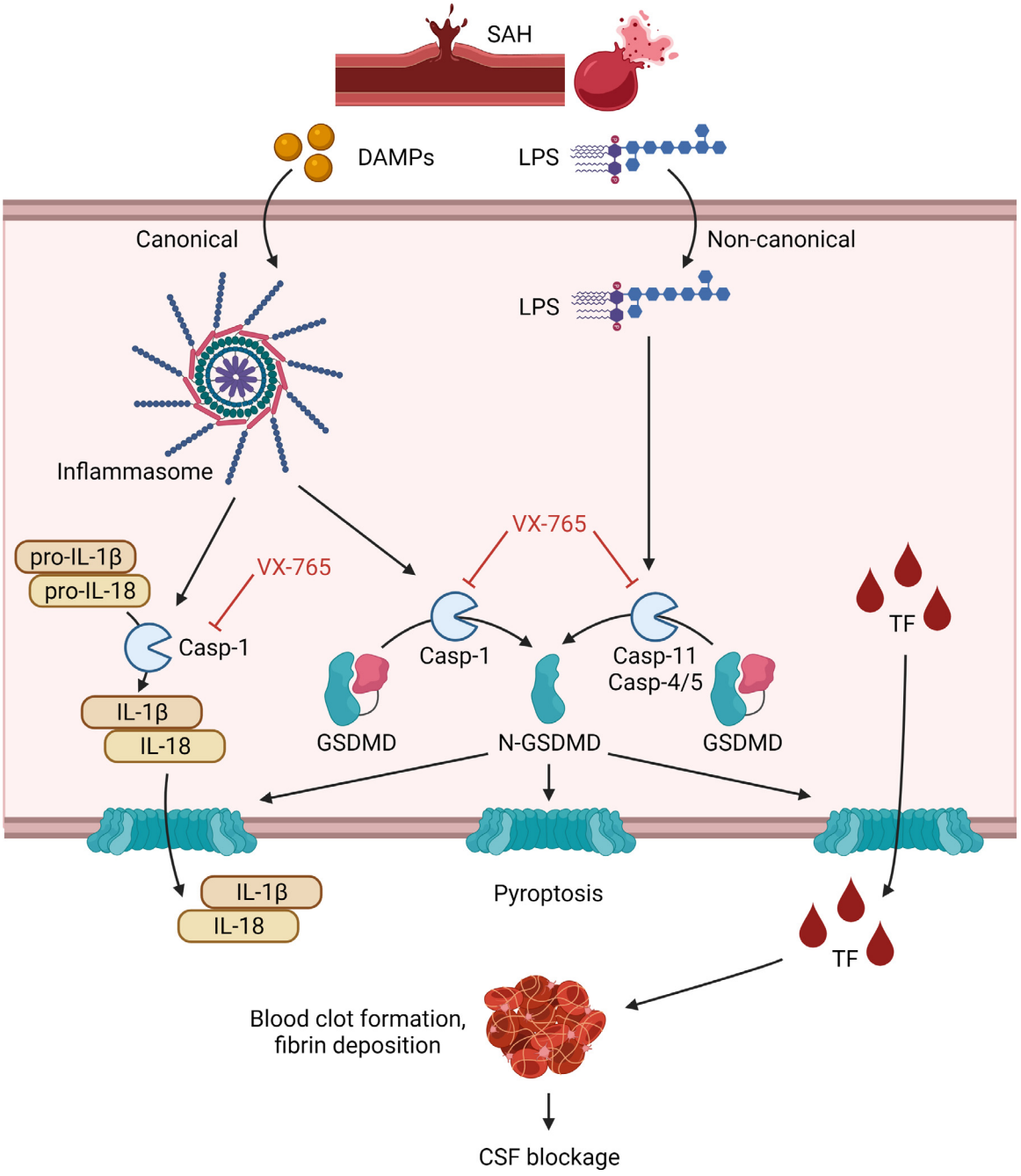

Figure 1. Proposed mechanism of VX-765 preventing pyroptosis and CSF blockage. Casp, caspase; CSF, cerebrospinal fluid; DAMPs, damage-associated molecular patterns; GSDMD, gasdermin D; IL, interleukin; LPS, lipopolysaccharide; N-GSDMD, N-terminal gasdermin D; SAH, subarachnoid haemorrhage; TF, tissue factor. Created with biorender.com.

caspase-1, IL-1, IL-18, and gasdermin protein levels at 72 h after SAH, which the authors confirmed by using gasdermin knockout mice. VX-765 treatment also improved long-term functional outcome, reduced post-SAH ventricular volumes as a measure of hydrocephalus, and reduced hippocampal neuronal loss. Finally, primary cell culture experiments revealed astrocytes as an important TF source.

The complex and diverse methods applied, the broad mechanistic insights and combination of clinical and experimental investigations underline the high translational value of the study. Nevertheless, some important questions are left unanswered. For instance, secondary cerebral ischaemia does not seem to play a major role in both models even though the volume of blood in the subarachnoid space and CSF is relatively large. This may point at a potential limitation of the applied models, and VX-765 effects may be investigated in additional models potentially including large animals in subsequent confirmative studies. Large animal models are expected to play an important role in translational research targeting cerebral haemorrhages and allow more thorough longitudinal assessment of treatment effects by brain imaging.[9,10]

Another point of discussion is that VX-765 is not only a potent inhibitor of caspase-1, but also of caspase-4/5 (Ki <0.6 nM),[5] which is caspase-11 in rodents. These caspases are non-canonical inflammasome elements activated by intracellular lipopolysaccharide and cannot cleave pro-IL-1β/pro-IL-18. However, they also induce the cleavage of gasdermin D resulting in pyroptosis,[6] which has been suggested to facilitate TF release.[7] Hence, the mechanism by which VX-765 reduces TF release and clotting and thereby preventing CSF blockage may also be due to inhibiting gasdermin D cleavage by caspases and thus abrogating pyroptosis (Figure 1). Other aspects awaiting investigation is the therapeutic time window of VX-765 treatment, optimal route and duration of administration, or proper dose translation for a future clinical approach. The latter is important because investigation of different doses, although performed by Fang *et al.*, was preliminary. A long-term investigation of the treatment's safety profile is also warranted. Finally, showing compatibility and increased benefit of a therapeutic strategy combining VX-765 therapy with established SAH treatments will be beneficial.

In summary, the presented work represents a major step in the direction of practical implementation of pharmacological support therapies targeting inflammation in SAH and may inform a series of targeted translational experiments finally cumulating in an early-stage clinical trial.

Declaration of interests

The authors do not declare any conflict of interests.

Author contributions

J.B. wrote the initial draft of the manuscript. M.Z. and N.P. provided invaluable comments and improvements. All authors revised and acknowledged the final manuscript.

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