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Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications

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Abstract

Focal cerebral ischaemia and post-ischaemic reperfusion cause cerebral capillary dysfunction, resulting in oedema formation and haemorrhagic conversion. There are substantial gaps in understanding the pathophysiology, especially regarding early molecular participants. Here, we review physiological and molecular mechanisms involved. We reaffirm the central role of Starling's principle, which states that oedema formation is determined by the driving force and the capillary "permeability pore". We emphasise that the movement of fluids is largely driven without new expenditure of energy by the ischaemic brain. We organise the progressive changes in osmotic and hydrostatic conductivity of abnormal capillaries into three phases: formation of ionic oedema, formation of vasogenic oedema, and catastrophic failure with haemorrhagic conversion. We suggest a new theory suggesting that ischaemia-induced capillary dysfunction can be attributed to de novo synthesis of a specific ensemble of proteins that determine osmotic and hydraulic conductivity in Starling's equation, and whose expression is driven by a distinct transcriptional program.

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Contributors

JMS originated the overall concept for this review and wrote the first and second drafts. TAK contributed to and helped edit the first and second drafts, and supplied important citations. MC participated in the original work on the sections on the $NC_{Ca\text{-}ATP}$ channel and contributed to the first draft. KVT did the computer analysis of the gene promoter regions. VG engaged in numerous intellectual exchanges with JMS during formulation of concepts for this review.

Conflict of interest

JMS and MC have applied for a US patent, "A novel non-selective cation channel in neural cells and methods for treating brain swelling" (application number 10/391,561).

Introduction

Dysfunction of cerebral capillaries due to ischaemia and post-ischaemic reperfusion results in a progressive alteration in the permeability of the blood–brain barrier, leading to formation of ionic oedema, vasogenic oedema, and haemorrhagic conversion. When capillaries that form the blood–brain barrier can no longer retain intravascular constituents, such as Na^+, water, serum proteins, and blood, these substances enter into the extracellular space of the brain and cause swelling. It is common to divide oedema into different subtypes,[1-3] but it is not typical to include haemorrhagic conversion in the same discussion. Yet, it now seems that ionic oedema, vasogenic oedema, and haemorrhagic conversion share important molecular antecedents, both pretranscriptional (ie, activation of transcription factors) and transcriptional, which suggests that haemorrhagic conversion may represent an endstage in a process that manifests initially as oedema.

Brain oedema and haemorrhagic conversion are important topics for neurologists and neurosurgeons who cope daily with their damaging consequences. There are excellent reviews on these subjects,[3-6] but our purpose here is different. We bring together definitions and ideas as they have developed over time, couple them with a modern understanding of physiological and molecular mechanisms, and unify what were previously considered to be distinct or conflicting theories on oedema formation and haemorrhagic conversion.

Critical features of cerebral oedema

Oedema versus swelling

Oedema is detrimental because it causes swelling (figure 1). Swelling means that the volume occupied by a given mass of tissue is increased—such as by a tumour, oedema, or blood. Swelling is harmful because of its effects on adjacent tissues, with these effects magnified by the fixed volume of the skull. Swollen tissues exert a mechanical force on the surrounding shell of tissue, displacing it and increasing tissue pressure within it. When tissue pressure exceeds capillary pressure, capillary inflow is compromised, leading to ischaemia, formation of oedema, and swelling of the shell.[7] Oedema and swelling are both indicators and causes of injury.

Swelling requires active blood flow

Swelling implies that a new constituent is added to the extracellular space of the brain. Excluding a tumour, the new constituent can only come from the vascular space. The absolute requirement for active blood flow is easily appreciated with a simple thought experiment. Excision of a piece of tissue from a live brain, whether in the operating room or laboratory, will cause the cells within the tissue to die, showing shifts in ionic and water content between extracellular and intracellular spaces that are characteristic of cytotoxic oedema. However, such tissues will not swell, will not become heavier, and will not show ionic oedema, vasogenic oedema, or haemorrhagic conversion, because there is no source of new water, ions, and blood. This thought experiment reinforces the distinction between cytotoxic oedema and the three pathophysiological processes (ionic oedema, vasogenic oedema, and haemorrhagic conversion), with the latter three requiring blood flow to cause swelling.

With post-ischaemic reperfusion, the requirement for active blood flow is fulfilled. In the case of unperfused tissue, there is a spatial gradient of ischaemia or hypoxia, ranging from profound hypoxia in the core, to near-critical hypoxia in the penumbra, to normoxia further away. These zones are associated with different molecular and physiological responses.[8] Ionic oedema forms in the zone of perfused but severely ischemic tissue. In a rodent model of malignant cerebral oedema 8 h after permanent middle cerebral artery occlusion (figure 1), oedema fluid is located mostly in viable regions adjacent to the core, with minimal excess water in the poorly-

perfused core.[9] MRI confirms that oedema is first found in peri-infarct regions that are perfused.[10]

Oedema fluid moves by bulk flow (convection) into the unperfused tissue. The driving force for this movement is the concentration gradient for the constituents that are moving, including Na^+ and Cl^-, and water. Before equilibration, areas within the core will contain little or no excess electrolytes, whereas penumbral areas adjacent to infarct will contain an excess of electrolytes and water. The rate of accumulation of excess Na^+ in the core may be used to estimate the age of the infarct.[11]

Starling's principle, oedema, and the ischaemic brain

Starling's principle

Over a century ago, Starling established the basic principle involved in the formation of oedema.[12] According to Starling, understanding oedema formation requires the identification of two features: the driving force, which "pushes" substances into the brain; and the permeability pore, which allows a transcapillary passage of these substances from the intravascular to the extracellular space.

The driving force is determined by the sum of hydrostatic and osmotic pressure gradients (figure 2). Hydrostatic pressure is determined by the difference between precapillary arteriolar and postcapillary venular pressures, which are affected by blood and tissue pressure. Osmotic pressure is determined by the concentration of osmotically active particles in blood versus extracellular tissues. In the normal brain, osmotic pressure plays a much more important part than hydrostatic pressure, due to the existence of tight junctions between endothelial cells that minimise this mechanism of fluid transfer across the capillary. Under pathological conditions, both osmotic and hydrostatic pressure gradients have critical roles in fluid transfer.

The second factor, the permeability pore, is determined by passages through and between the capillary endothelial cells that form the blood–brain barrier.[13] Passages through endothelial cells can be formed by ion channels, if those channels are expressed on both luminal and abluminal sides of endothelial cells. Also, reverse pinocytosis has been put forth as a mechanism by which substances can undergo transcapillary movement. Formation of passages between capillary endothelial cells implies either that cells contract, partially "retracting" cell borders, that cells loose tight junctions between themselves, or that the cells are totally lost—eg, by necrotic death.

Cytotoxic oedema

Cytotoxic oedema is a premorbid process that involves oncotic swelling of cells due to movement of osmotically active molecules (principally Na^+, Cl^-, and water) from the extracellular to the intracellular space.[14-17] The terms cytotoxic oedema, cellular oedema, oncosis, and necrotic volume increase are synonymous and refer to pathophysiological processes at the cellular level. With cytotoxic oedema, there is no new constituent from the intravascular space added and tissue swelling does not occur. However, cytotoxic oedema creates the driving force for transcapillary formation of ionic and vasogenic oedema, which do cause swelling.

An older definition of cytotoxic oedema encompassed not only the definition as given here, involving a strictly cellular disturbance, but also the concept of transcapillary water and electrolyte transport into brain parenchyma—ie, ionic oedema. Because distinct physiological processes are involved, however, we regard it as important to maintain independent definitions.

Movements of osmotically active molecules into the cell can occur either by primary active transport or secondary active transport. Primary active transport (ATP-dependent, Na^+,K^+ ATPase, etc) requires continuous expenditure of energy, which is not readily available during the conditions of ischaemia.[18,19] Secondary active transport uses energy stored in pre-existing ionic gradients across the cell membrane (ion channels, $Na^+/K^+/Cl^-$ cotransporters, etc). Because there is a dysfunctional energy state that exists during ischemia, in this Review we focus on mechanisms that are largely independent of continuous expenditure of energy.

Two types of substances are involved in cytotoxic oedema, primary drivers and secondary participants. Primary drivers are molecules that are more concentrated outside the cell than inside of the cell and that are normally extruded from the cell by primary active transport. Secondary participants are molecules for which no pre-existing electrochemical gradient normally exists, but for which a gradient is created by the primary drivers. If Na^+ is the primary driver, Cl^- and water would be the secondary participants that move in order to maintain electrical and osmotic neutrality. Many types of Cl^- channels normally exist in all cells of the CNS. Aquaporin channels that may aid bulk flow of water are up-regulated, at least in astrocytes, in CNS ischaemia.[20,21]

Different molecular mechanisms can be used for secondary active transport. For Na^+, conventional thinking asserts that in neurons and astrocytes, constitutively expressed Na^+ influx pathways, including tetrodotoxin-sensitive Na^+ channels, $Na^+/K^+/Cl^-$ co-transporters, or N-methyl-D-aspartate receptor channels can admit Na^+ during the course of normal activity or during pathological depolarisation[22-24] and during ischaemia, newly admitted Na^+ cannot be extruded due to failure of Na^+ or K^+, ATPase, and other ATP-dependent transporters.[25]

Apart from constitutively expressed pathways, non-selective cation channels up-regulated by ischaemia or oxidative stress may provide new pathways for Na^+ influx. Transient receptor potential channels[26] and the sulfonylurea receptor 1 (SUR1)-regulated $NC_{Ca\text{-}ATP}$ channel[9,27,28] can act in this manner. The $NC_{Ca\text{-}ATP}$ channel is transcriptionally up-regulated within 2 −3 h of ischaemia (figure 3). Opening of this channel, which is triggered by ATP depletion, causes cell depolarisation, cell blebbing (figure 4), cytotoxic oedema, and oncotic cell death (figure 5), all of which are prevented by blocking the channel.

Opening non-selective cation channels allows K^+ to leave the cell, but movements of Na^+ and K^+ do not neutralise one another, because the cell is full of negatively charged proteins and other macromolecules that act to bind K^+,[4] resulting in a substantially greater inflow of Na^+ than outflow of K^+. The net inflow of Na^+ generates an osmotic force that drives an influx of water, which is typical of cytotoxic oedema.

Cytotoxic oedema is tied to cell death. With the inflow of Na^+ down its concentration gradient, and the resultant inflow of Cl^- and water, the cell depolarises, blebs or outpouchings form in the cell membrane, and eventually the membrane ruptures as the cell undergoes lysis—necrotic cell death (figure 5).[29,30]

Cytotoxic oedema (oncotic volume increase) may be contrasted with “apoptotic volume decrease”.[31] The former involves influx of Na^+, Cl^-, and water, whereas the latter involves the opening of K^+ selective channels, which results in K^+ efflux and is accompanied by Cl^- efflux and loss of water from the cell. Volume descrease with apoptosis results in cell shrinkage, which presages apoptotic cell death.

Driving force for oedema formation

The extracellular space of the brain is small compared with the intracellular space. The extracellular space constitutes of only 12−19% of brain volume.[16] The movement of ions and

water into cells during the formation of cytotoxic oedema results in depletion of these constituents from the extracellular space.[32,33] Cytotoxic oedema sets up a new gradient for Na^+, now across the blood–brain barrier, between the intravascular space and the extracellular space, which acts as a driving force for transcapillary movement of oedema fluid. If neurons and astrocytes undergo necrotic death, joining their intracellular contents to that of the extracellular space, a concentration gradient for Na^+ is still set up across the blood–brain barrier, because the extracellular space of the brain is smaller than the intracellular space, as indicated by the high K^+ concentrations and low Na^+ concentrations of normal homogenised brain tissue[4]—coupled with the fact that K^+ ions remain largely bound to negatively charged intracellular proteins and other macromolecules.[4] Thus, whether or not cells are intact, cytotoxic oedema and cell death create a transcapillary gradient that acts to drive subsequent movement of oedema fluid.

Permeability pores

In accordance with Starling's principle, the driving force across the blood–brain barrier that is newly created by cytotoxic oedema represents a form of potential energy that will not be released unless the permeability properties of the blood–brain barrier are changed. Later in this Review we consider the permeability pores that permit fluxes down concentration gradients across the capillary wall. Ischaemia-induced changes in capillary permeability can be organized into three distinct phases (ionic oedema, vasogenic oedema, and haemorrhagic conversion), based on the principal constituents that undergo transcapillary movement (figures 2 and 5). The three phases are thought to occur sequentially, but the likelihood and rapidity of transition from one phase to another probably depends on factors such as duration and depth of hypoxia during perfusion or prior to reperfusion. Thus, the reperfused capillary in the core that was completely ischaemic is more likely to go on to the third phase than the hypoxic capillary at the edge of the penumbra.

First phase: formation of ionic oedema—The earliest phase of endothelial dysfunction in ischaemia is characterised by the formation of ionic oedema (figures 2 and 5).[2,4,34-36] Formation of ionic oedema involves transport of Na^+ across the blood–brain barrier, which generates an electrical gradient for Cl^- and an osmotic gradient for water, thus replenishing Na^+, Cl^- and water in the extracellular space that was depleted by formation of cytotoxic oedema. As with cytotoxic oedema, in ionic oedema, the amount of Na^+ accumulated exceeds the amount of K^+ lost, giving a net inflow of Na^+ into oedematous brain.[4,35]

Formation of ionic oedema is clearly distinct from the formation of vasogenic oedema because it involves abnormal Na^+ transport when there is normal exclusion of protein by the blood–brain barrier.[37-40] Early water influx (stage of ionic oedema) correlates with Na^+ accumulation and precedes albumin influx (stage of vasogemic oedema) by 6 h or more. In this phase of ionic oedema, the blood–brain barrier remains “intact”—ie, macromolecules do not permeate it. Thus, influx of Na^+ cannot be accounted for by leaking from the blood–brain barrier, reverse pinocytosis, loss of tight junctions, or other physical processes that would also allow transport of serum macromolecules along with Na^+.

As with cytotoxic oedema, two mechanisms can account for selective flux of Na^+ across the blood–brain barrier, primary active transport and secondary active transport, but again, we only focus on secondary active transport mechanisms that depend on preexisting electrochemical gradients. Unlike neurons and astrocytes, endothelial cells do not express voltage-dependent channels that conduct Na^+.[41] They express ligand-gated channels that could act in this manner,[41] but there is no evidence to show their involvement.

The secondary active $Na^+/K^+/Cl^-$ co-transporter,[42] located mostly on the luminal side of the endothelium, may be involved in the formation of ionic oedema on the basis of salutary effects

of preischemic administration of the co-transporter inhibitor bumetanide.[43] However, this mechanism requires abluminal Na^+/K^+ ATPase to complete transcapillary flux of Na^+.[43] Thus, invoking this mechanism in the context of ischaemia is problematic, although it may be relevant should energy restoration occur with timely reperfusion.

Data from our laboratory implicate SUR1-regulated $NC_{Ca\text{-}ATP}$ channels in the formation of ionic oedema (figure 3). Post-ischaemic block of the channel by low-dose glibenclamide reduces oedema by 50%.[9] Involvement of $NC_{Ca\text{-}ATP}$ channels implies that the formation of ionic oedema does not co-opt existing membrane proteins, but instead requires the expression of a new protein by endothelial cells of ischaemic but perfused capillaries.

A mechanism that involves Na^+-conducting channels in transcapillary flux of Na^+ is analogous to cytotoxic oedema of endothelial cells. Channels on the luminal side contribute to cytotoxic oedema of endothelial cells, providing an influx pathway for Na^+, whereas channels on the abluminal side act to relieve this cytotoxic oedema by providing an efflux pathway for Na^+ down its concentration gradient from the cell into extracellular space. Obviously, this relief mechanism completes the pathway for transcapillary flux of Na^+. As noted previously, Cl^- and water follow via their own respective channels, completing the process of formation of ionic oedema. Although Cl^- channels are present,[41] expression of aquaporin channels by endothelium remains to be clarified, with aquaporin-1 but not aquaporin-4 possibly playing a role in ischaemia.[44]

In this stage of ionic oedema, the integrity of the blood–brain barrier is maintained, capillary tight junctions are preserved, and macromolecules are excluded from brain parenchyma. Thus, the driving force for the formation of oedema is determined only by osmotic pressure gradients, with hydrostatic pressure gradients being essentially irrelevant (figure 2).

Second phase: formation of vasogenic oedema—The second phase of endothelial dysfunction is characterised by the breakdown of the blood–brain barrier, with leakage of plasma proteins into extracellular space (figures 2 and 5). Macromolecules such as albumin, IgG, and dextran, to which the blood–brain barrier is normally impermeable, now pass readily across the endothelial barrier.

Vasogenic oedema may be considered as an ultrafiltrate of blood,[45,46] which suggests that the permeability pore is now quite large. The permeability pore that allows the passage of larger molecules across the blood–brain barrier has not been uniquely identified, and may have contributions from more than one mechanism. Any physical disruption of the capillary must be relatively limited, however, to account for egress of a proteinacious ultratrafiltrate without passage of erythrocytes.

Several mechanisms have been proposed to account for changes in permeability that give rise to vasogenic oedema, including reverse pinocytosis,[47] disruption of Ca^{2+} signalling,[48] actin polymerisation-dependent endothelial cell rounding or retraction with formation of interendothelial gaps, uncoupling of tight junctions, and enzymatic degradation of basement membrane. Formation of interendothelial gaps is reported with many inflammatory mediators, [49] including mediators up-regulated in cerebral ischaemia such as thrombin.[50] Thrombin-induced endothelial cell retraction may account for vasogenic oedema associated not only with focal ischaemia but also with intracerebral haematoma.[51,52] Uncoupling of tight endothelial junctions is reported after the up-regulation of vascular endothelial growth factor (VEGF), which increases hydraulic conductivity in isolated perfused microvessels, increases vascular permeability, and promotes formation of oedema.[53] Antagonism of VEGF reduces oedema associated with post-ischemia reperfusion.[54] Degredation of basement membrane required for structural integrity of capillaries is observed with enzymes that are up-regulated in cerebral

ischaemia, especially the matrix metalloproteinases (MMP), MMP-9 (gelatinase B), and MMP-2 (gelatinase A; figure 2).[55-58] Ischaemia activates latent MMPs and causes de novo synthesis and release of MMPs.[55,59,60] MMP inhibitors reduce ischaemia or reperfusion-associated brain oedema.[61,62] Other proteins that are up-regulated, and whose function results in degradation of the blood–brain barrier, include nitric oxide synthase (NOS), either inducible NOS[63] or neuronal NOS.[64] Notably, these various molecular mechanisms establish the important concept that constitutively expressed participants may play only a limited part, and up-regulation of a family of proteins that change the permeability of the blood–brain barrier may well be the norm.

Once the integrity of the blood–brain barrier is lost, capillaries behave like fenestrated capillaries, and both the hydrostatic and osmotic pressure gradients contribute to oedema formation (figure 2). Determinants of hydrostatic pressure, including systemic blood pressure and intracranial pressure, now assume an important role. Determinants of osmotic pressure now consist of all osmotically active molecules, including Na^+ and macromolecules. However, there are implications regarding clinical management: systemic blood pressure must be sufficient to perfuse the brain, but excess pressure will promote oedema formation;[65] and intracranial pressure, which determines tissue pressure, must be lowered to appropriate levels, but lowering it too much will promote oedema formation. Optimisation of parameters to achieve these conflicting goals is difficult. Treatments generally include use of osmotically active drugs such as mannitol, but their effects may only be transiently beneficial.

These concepts shed light on why there can be mixed outcomes after decompressive craniectomy[66,67]—a procedure that abruptly lowers tissue pressure. In the stage of ionic oedema, hydrostatic pressure and therefore tissue pressure is unimportant for oedema formation. By contrast, in the stage of vasogenic oedema, tissue pressure is a critical determinant of oedema formation. Decompressive craniectomy may be safe if done early—ie, during the stage of ionic oedema when the blood–brain barrier is intact—because it may aid in restoring reperfusion by reducing intracranial pressure. By contrast, if decompressive craniectomy is done later—ie, during the stage of vasogenic oedema—it will decrease tissue pressure, drive formation of vasogenic oedema, and thus may have an unintended deleterious effect.[68] Brain imaging may guide the timing of treatment by detecting these stages. Diffusion restriction on MRI correlates with the cytotoxic stage,[10] whereas early hypodensity before mass effect on computed tomography scans may be useful to assess ionic versus vasogenic oedema before decompressive craniectomy.[69,70]

Third phase: haemorrhagic conversion—The third phase of endothelial dysfunction is marked by catastrophic failure of capillary integrity, during which all constituents of blood, including erythrocytes, extravasate into the brain parenchyma (figures 5 and 6). Up to 30−40% of ischaemic strokes undergo spontaneous haemorrhagic conversion, a complication that is more prevalent and severe with use of thrombolytic stroke therapy.[71-73] Haemorrhagic conversion—the transformation of a bland infarct into a haemorrhagic infarct after restoration of circulation—accounts for a major cause of early mortality in patients with acute stroke—about 26−154 extra deaths per 1000 patients.[74-78]

Prolonged ischaemia, aggravated by reperfusion, causes initial dysfunction and later death of capillary endothelial cells.[79-81] As this process develops, the blood–brain barrier is increasingly compromised, capillaries begin to leak, and eventually they lose their physical integrity. Eventually the capillaries will no longer contain circulating blood, resulting in the formation of petechial haemorrhages–hemorrhagic conversion. The close association between the blood–brain barrier compromise and haemorrhagic conversion is supported by both animal[69] and human studies.[70,82,83] These studies show that haemorrhagic conversion after thrombolytic therapy can be predicted on the basis of pre-existing dysfunction of the blood–

brain barrier manifested either as gadolinium enhancement or hypodensity on computed tomographic imaging.

Haemorrhagic conversion is probably a multifactorial problem due to reperfusion injury and oxidative stress. Mechanisms may include plasmin-generated laminin degradation, endothelial cell activation, transmigration of leucocytes through the vessel wall, and other processes.[80,84] Other factors listed above in relation to vasogenic oedema may also be important. Exogenous VEGF when given intravascularly soon after reperfusion aggravates haemorrhagic transformation.[85] Dysregulation of extracellular proteolysis plays a key part in haemorrhagic transformation, with MMPs being critical participants.[58,84,86,87] As with vasogenic oedema, inhibition of proteolysis in the blood–brain barrier reduces haemorrhagic conversion with reperfusion.[61,62] Finally, oncotic death of endothelial cells, mediated by SUR1-regulated $NC_{Ca\text{-}ATP}$ channels, may also give rise to haemorrhagic conversion (figures 5 and 6). Additional research will be needed to determine the relative contribution of these various mechanisms, and to uncover new ones that may be involved.

Everything mentioned above, for the fenestrated capillary associated with vasogenic oedema, also holds in the haemorrhagic conversion phase. Theoretically, adding blood into the parenchyma and thereby increasing tissue pressure may reduce the hydrostatic driving force, but it does so at an untenable cost to the organ by: adding mass that contributes to increased intracranial pressure; adding the toxic oxidant, haemoglobin; and by inciting a robust inflammatory response, all of which contribute adversely to the outcome.[88-90] Implications for clinical management are similar to those for the previous stage, but optimising parameters to achieve the conflicting goals is now more difficult.

Energy considerations

The phases of oedema formation depicted here are grounded on physiological principals described over a century ago. These mechanisms account for massive fluxes of ions and water into brain parenchyma, despite the severe energy constraints typically encountered with ischaemia. During the formation of ionic oedema, the movement of ions and water occur by secondary active transport mechanisms, powered by concentration gradients originally formed by exclusion of Na^+ from neurons and astrocytes. During formation of vasogenic oedema, as well as during hemorrhagic conversion, movements of plasma and blood into parenchyma are driven by hydrostatic pressure generated by the heart. Thus, vast quantities of ions, macromolecules, water, and blood can move into the parenchyma with no new energy expenditure by the brain.

On the other hand, this accounting for movements of oedema fluid requires new protein synthesis induced by ischaemia in order to change the permeability of the blood–brain barrier. One important example is aquaporin-4 (Aqp4), now strongly implicated in ischemia-induced oedema.[20,91] As for the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel, which seems to be integral to the formation of ionic oedema, the need for protein synthesis has been shown at least for the SUR1 regulatory subunit of this channel, which is transcriptionally up-regulated in ischaemia[9]. In addition, the need for protein synthesis is true for prothrombin,[92,93] MMP-9,[55,56,94] VEGF,[95] and iNOS, which have important roles in vasogenic oedema and haemorrhagic conversion. New protein synthesis may require limited, perhaps “one-time” energy expenditure—which may ultimately be the last such expenditure on the way to self destruction of capillaries. Notably, the burden for new protein synthesis is mostly left largely to endothelial cells in capillaries that are still perfused, and thus most likely to maintain a positive energy balance the longest in the face of an ischaemic insult.

Transcriptional program

What links the various proteins, newly synthesised by ischemic endothelium, that are tied to progressive capillary dysfunction? Because the three phases of capillary dysfunction arise from a severe hypoxic insult, with or without free radicals generated upon reperfusion, synthesis of these proteins must be regulated by a transcriptional program that involves hypoxia or redox-sensitive transcription factors such as activator protein-1 (AP-1) [dimers of Fos, Jun and related oncoproteins that activate immediate early genes (IEGs)[96]], hypoxia inducible factor-1 (HIF-1), Sp-1 and nuclear factor-κB (NF-κB). Each of these factors is activated by focal cerebral ischaemia.[9,97-102] HIF is activated when oxygen tension falls below 5% (40 mmHg), and is progressively activated with a decrease in oxygen tension down to 0·2–0·1% (1·6–0·8 mmHg), close to anoxia.[103] Analysis of the promoter regions of the various proteins reveals the presence of one or more putative binding sites for each of these transcription factors (figure 7). Definitive evidence for involvement of all four factors in transcriptional regulation of proteins involved in cerebral oedema has not been published, but some gaps in knowlegde have been filled in,[104,107] including for Aqp4 (AP-1, Sp-1),104 SUR1 (Sp-1),[9,105,106] prothrombin (Sp-1),107 VEGF (Sp-1, HIF-1, AP-1)[108-111] and MMP-9 (NF-κB).[60,112]

Future research will add new details to the transcriptional program proposed here by identifying other hypoxia or redox-activated transcription factors involved. Nevertheless, the functional grouping of these four factors affirms the concept of a transcriptional program which, when unleashed, initiates a sequential dynamic alteration in the characteristics of the blood–brain barrier that can lead to demise of the organ and ultimately, demise of the organism.

Maladaptation or restructuring

At first glance, it may seem counterintuitive that evolution would favour a program that involves the transcriptional up-regulation of gene products that result in dysfunction and death of endothelial cells that form the blood–brain barrier. From a broader perspective, however, the scheme of events outlined here represents the first step in a larger program that involves revascularisation and recovery from ischaemic injury—as in many human endeavors, rebuilding of injured brain cannot begin until previous structures have been dismantled and removed. Thus, MMPs degrade neurovascular matrix, injure the blood–brain barrier, and thereby promote oedema and haemorrhage early on, but they are mandatory for later neurovascular remodelling and neuronal recovery.[113] VEGF promotes vasogenic oedema early on, but is critical to later angiogenesis.[114,115] SUR1-regulated $NC_{Ca\text{-}ATP}$ channels are involved in the oncotic death of CNS cells after ischaemia,[9] but this may be essential to the irrevocable breaking down of damaged tissues and inciting an inflammatory response that will hasten the clearing of debris in preparation for revascularisation and reconstruction.

Conclusion

All aspects of oedema formation and haemorrhagic conversion discussed in this brief review require further characterisation at the molecular, cellular, and organ levels, and futher studies will be needed to advance clinical therapies. Our understanding of cerebral capillaries is in its infancy, with progress hampered by their complex function, which cannot be duplicated in vitro. Nevertheless, there has been substantial progress in understanding the mechanisms of cerebral capillary dysfunction induced by ischaemia or reperfusion.

In summary, concepts originally articulated in the 19th century continue to inform our understanding of cerebral capillary function in the 21st century, making Starling's principle a cornerstone for understanding oedema formation in the brain. The driving force required for movement of fluids is largely independent of expenditure of new energy by the ischaemic brain. A unique transcriptional program may account for the progressive alterations in the

permeability of the blood–brain barrier that occur after ischemia or reperfusion. There are different, sometimes, competing theories on oedema—eg, vasogenic versus ionic oedema[4]—that can be unified and joined to the seemingly disparate concept of haemorrhagic conversion, resulting in a rational framework that accounts for the continuum of pathological deterioration of cerebral capillaries by ischemia or reperfusion.

Strategy and search selection criteria

References for this review were identified by searches of PubMed from 1980 to September 2006 and from citations listed in reviewed articles. Search terms used were “cerebral ischemia”, “stroke”, “brain swelling”, “cytotoxic edema”, “ionic edema”, “vasogenic edema”, “hemorrhagic conversion”, “decompressive craniectomy”, “capillary”, “endothelium”, “blood brain barrier”, “Starling's principal”, “cell death”, “apoptosis”, “necrosis”, “sodium”, “ion channels”, “tight junctions”, “adenosine triphosphate”, “transcription factors”, “aquaporin”, “sulfonylurea receptor 1”,”thrombin/prothrombin”, “vascular endothelial growth factor”, “matrix metalloproteinase”, “endothelial nitric oxide synthase”,”inducible nitric oxide synthase”, “activator protein-1”, “hypoxia inducible factor-1”,”sp-1”,and ”nuclear factor-kB”. Only papers published in English were reviewed. The final reference list was generated based on relevance, originality, journal impact factor, and space limitations.

The conceptualisation of oedema and haemorrhagic conversion depicted here, with transcriptional events recognised for their central role, affords new hope for advances in therapy. Involvement of new protein synthesis in ischaemic perfused capillaries means that drug delivery is feasible and a window of time is available. This bodes well for future development of pharmaceutical and molecular strategies to target involved proteins and genes. This conceptualisation also implies that targeting a single gene or gene product may not be sufficient, if the transcriptional program generates multiple proteins with redundant or synergistic biological effects. Nevertheless, progress is inevitable, giving real hope that secondary brain injury resulting from ionic oedema, vasogenic oedema, and haemorrhagic conversion will soon yield to appropriately directed therapies.

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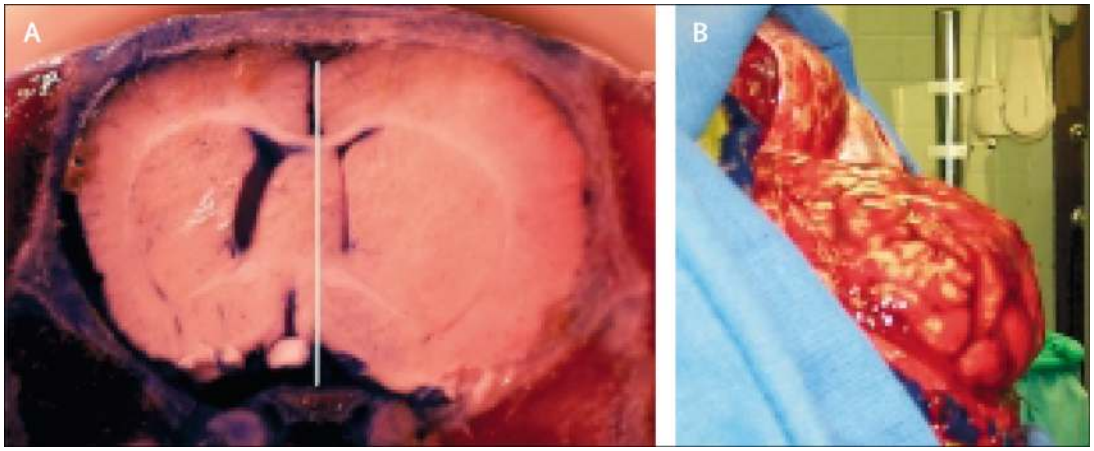

Figure 1. Brain swelling after middle cerebral artery occlusion

Left: photograph of coronal section of rat head following middle cerebral artery occlusion; post-mortem perfusion with Evans blue and India ink shows regions with persistent circulation (darker areas, left) versus regions without appreciable circulation (pink area, right); white line from the superior sagittal sinus to the clivus indicates the midline, showing extensive shift due to massive swelling of the involved hemisphere. Right: intraoperative photograph showing massive brain swelling causing herniation of the brain out of the skull following decompressive craniectomy.

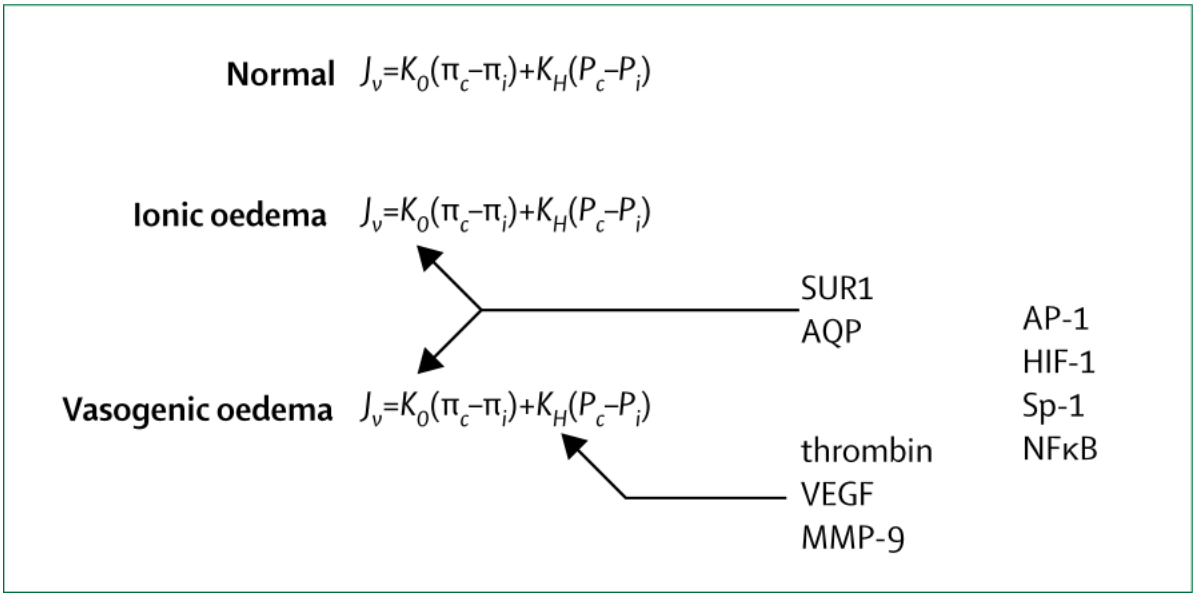

Figure 2. Starling's equation, classically stated as $J_v = K_f[(P_c - P_i) - (\pi_c - \pi_i)]$, describes capillary permeability under normal and pathological conditions

Formulated in 1896 by the British physiologist Ernest Starling, the Starling equation describes the role of hydrostatic and osmotic forces in the movement of fluid across capillary endothelial cells. According to the equation, the movement of fluid depends on five variables: capillary hydrostatic pressure (P_c), interstitial hydrostatic pressure (P_i), capillary osmotic pressure (π_c), interstitial osmotic pressure (π_i), and a filtration coefficient (K_f). Here, two distinct "filtration" coefficients, the hydraulic conductivity (K_H), and the osmotic conductivity (K_O), are used to describe the situation in brain capillaries. The equation gives the net filtration or net fluid movement (J_v), with outward force being positive, meaning that fluid will tend to leave the capillary. The filtration coefficients, K_H and K_O, determine oedema formation. Normally, values of K_O and K_H are small or close to zero, and no oedema forms. With ionic oedema, $K_O \geq 0$ and $K_H \approx 0$, with the change in K_O due to upregulation of Na+ flux pathways, such as the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel and possibly aquaporin (AQP) channels. With vasogenic oedema, $K_O \geq 0$ and $K_H \geq 0$, with the increase in K_H being due to upregulation of prothrombin, VEGF and MMP-9. Upregulation of various oedema-associated proteins can be attributed, at least partly, to activation of a transcriptional program involving AP-1, HIF-1, Sp-1 and NF-κB. Note that the driving forces for fluid movement are not generated by the ischaemic brain; rather, hydrostatic pressure, P, is generated by the heart, and osmotic pressure, π, arises from potential energy stored in electrochemical gradients established before onset of ischaemia.

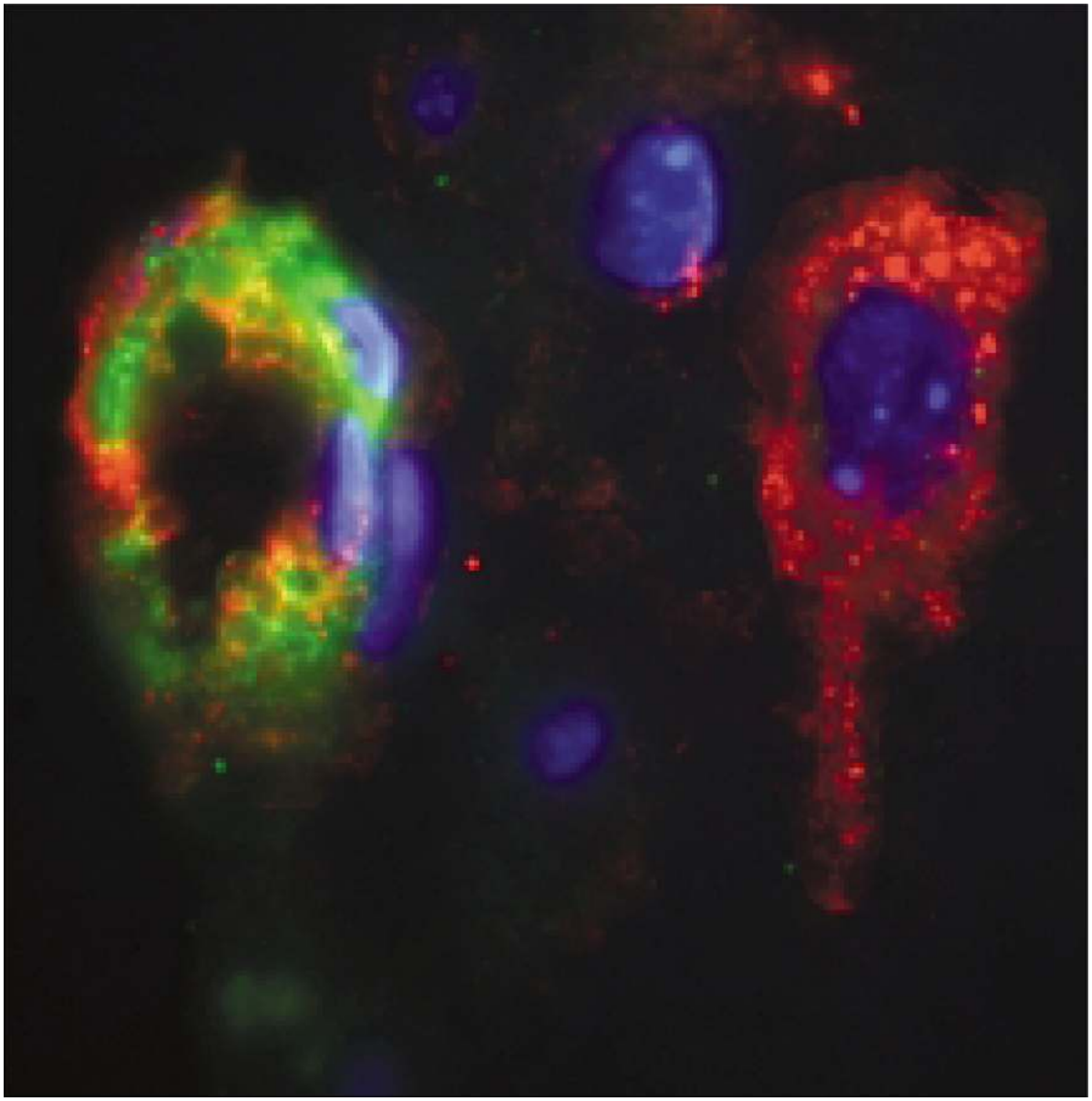

Figure 3. SUR1, the regulatory subunit of the $NC_{Ca\text{-}ATP}$ channel, is up-regulated in focal cerebral ischemia, as shown in rat tissue

Brain tissue from the core of an infarct 6 h after middle cerebral artery occlusion. Capillary (left) labelled for von Willebrand factor (green) and for SUR1 (red), next to a dying neuron with blebs (right) that labels strongly for SUR1 (red); nuclei labeled with DAPI (blue).

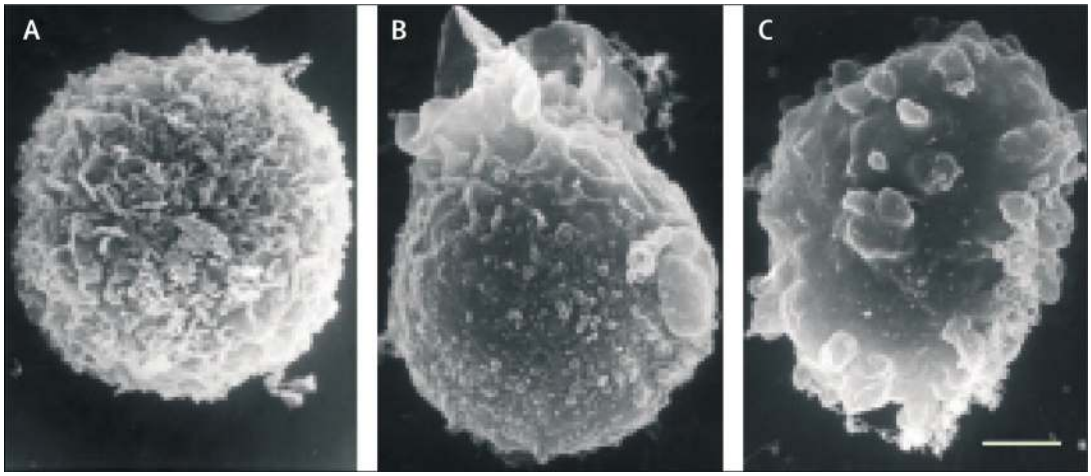

Figure 4. Cell blebbing after NaN_3-induced ATP depletion

Scanning electron micrographs of freshly isolated native reactive astrocytes from adult rat brain. Formaldehyde-glutaraldehyde fixation was initiated under control conditions (A), 5 min after exposure to 1 mM NaN_3 (B), and 25 min after exposure to 1 mM NaN (C). Bar, 12 μm. Reproduced with permission from Chen and Simard.[27]

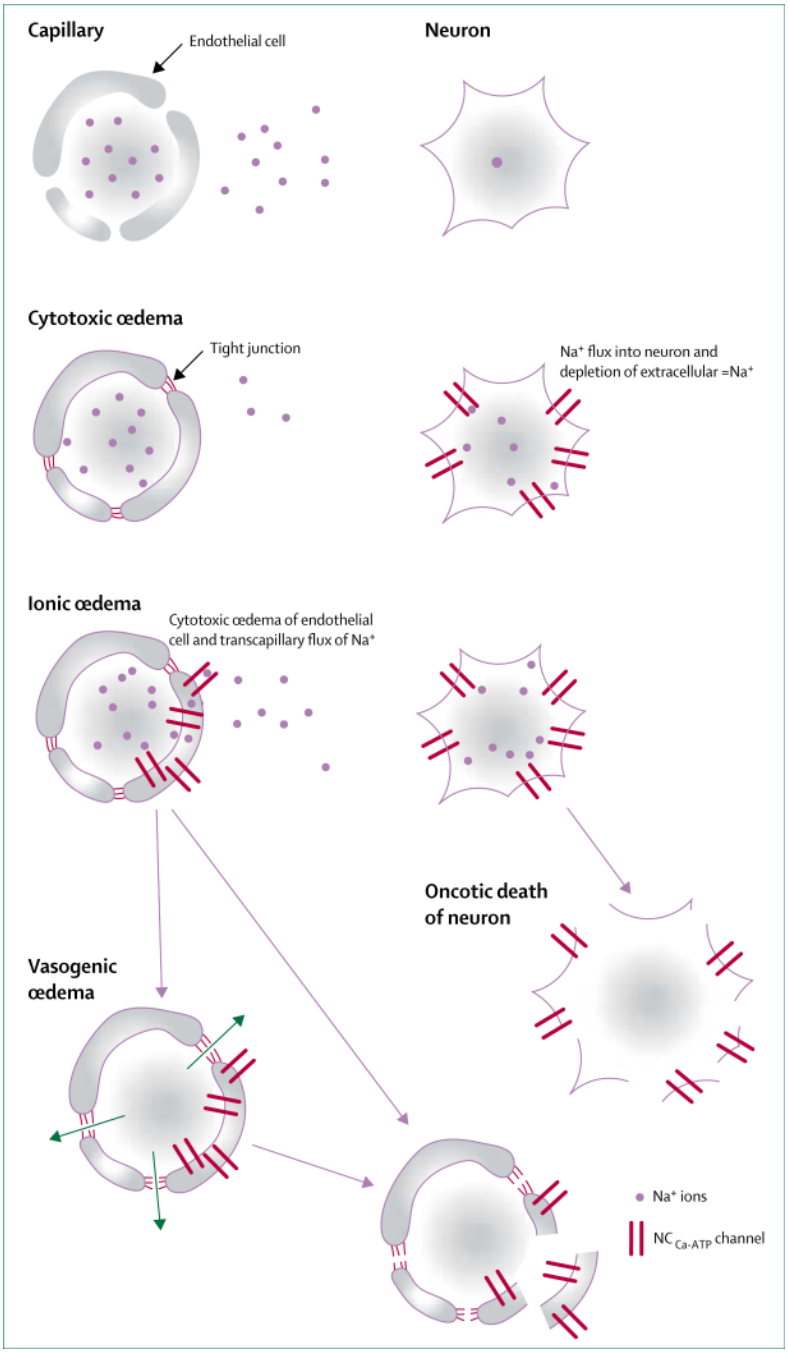

Figure 5. Schematic diagram illustrating various types of edema progressing to hemorrhagic conversion

Normally, Na^+ concentrations in serum and in extracellular space are the same, and much higher than inside the neuron. Cytotoxic oedema of neurons is due to entry of Na^+ into ischaemic neurons via pathways such as $NC_{Ca\text{-}ATP}$ channels, depleting extracellular Na^+ and thereby setting up a concentration gradient between intravascular and extracellular compartments. Ionic oedema results from cytotoxic oedema of endothelial cells, due to expression of cation channels on both the luminal and abluminal side, allowing Na^+ from the intravascular compartment to traverse the capillary wall and replenish Na^+ in the extracellular space. Vasogenic oedema results from degradation of tight junctions between endothelial cells,

transforming capillaries into “fenestrated” capillaries that allow extravasation (outward filtration) of proteinatious fluid. Oncotic death of neuron is the ultimate consequence of cytotoxic edema. Oncotic death of endothelial cells results in complete loss of capillary integrity and in extravasation of blood—ie, haemorrhagic conversion.

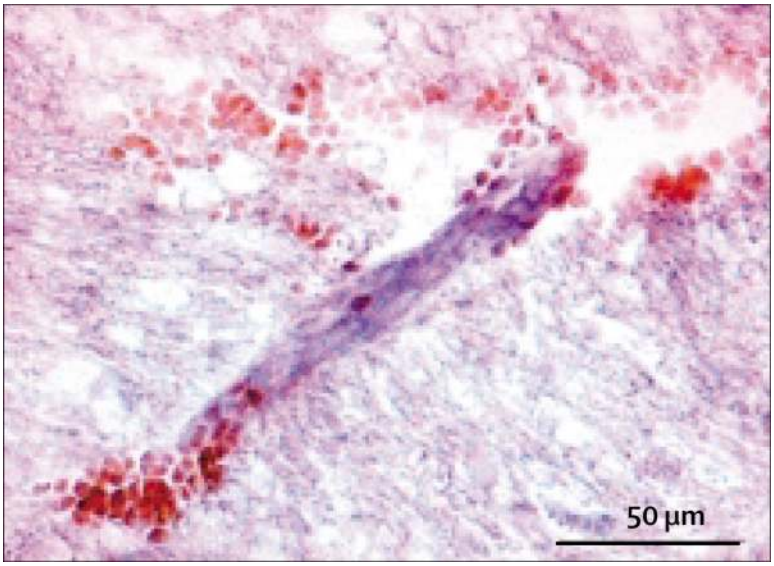

Figure 6. Haemorrhagic conversion with petechial haemorrhage is associated with transcriptional upregulation of sulfonylurea receptor 1 (SUR1) in ischaemic CNS tissues

In situ hybridisation for SUR1 (purple) shows strong labelling with antisense probe in a microvessel surrounded by extravasated erythrocytes (red).

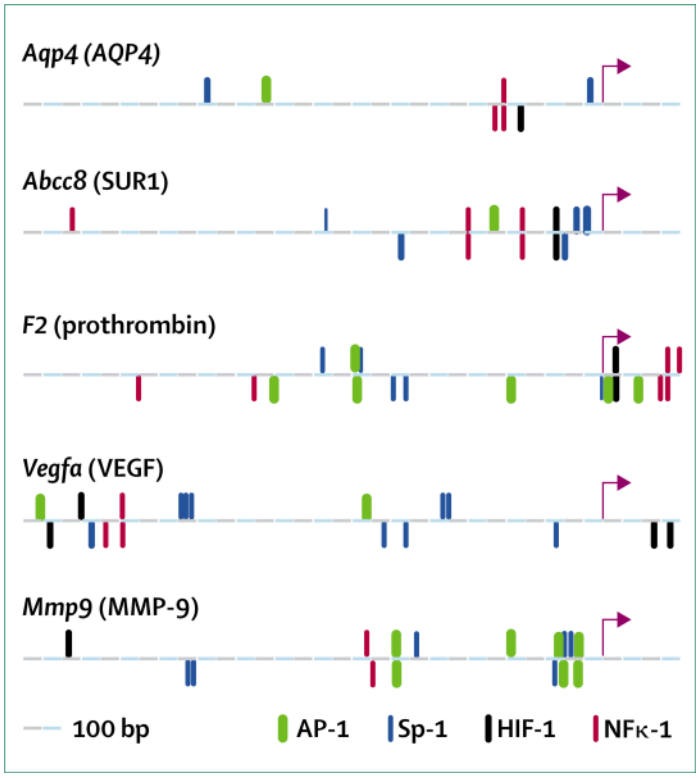

Figure 7. A distinct transcriptional program may account for sequential changes in ischaemia-induced changes in blood–brain permeability

The promoter regions of five genes (italicised) for proteins (in parentheses) involved in oedema formation, were analysed for potential consensus sequence binding sites for the transcription factors AP-1, Sp-1, HIF-1, and NF-κB. The predicted location of these putative binding sites on each promoter region is shown, with binding sites on the positive and negative strands indicated by upward and downward symbols, respectively.