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REVIEW

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Pathophysiology of ischaemic stroke: insights from imaging, and implications for therapy and drug discovery

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Preventing death and limiting handicap from ischaemic stroke are major goals that can be achieved only if the pathophysiology of infarct expansion is properly understood. Primate studies showed that following occlusion of the middle cerebral artery (MCA)—the most frequent and prototypical stroke, local tissue fate depends on the severity of hypoperfusion and duration of occlusion, with a fraction of the MCA territory being initially in a 'penumbral' state. Physiological quantitative PET imaging has translated this knowledge in man and revealed the presence of considerable pathophysiological heterogeneity from patient to patient, largely unpredictable from elapsed time since onset or clinical deficit. While these observations underpinned key trials of thrombolysis, they also indicate that only patients who are likely to benefit should be exposed to its risks. Accordingly, imaging-based diagnosis is rapidly becoming an essential component of stroke assessment, replacing the clock by individually customized management. Diffusion- and perfusion-weighted MR (DWI-PWI) and CT-based perfusion imaging are increasingly being used to implement this, and are undergoing formal validation against PET. Beyond thrombolysis *per se*, knowledge of the individual pathophysiology also guides management of variables like blood pressure, blood glucose and oxygen saturation, which can otherwise precipitate the penumbra into the core, and the oligaemic tissue into the penumbra. We propose that future therapeutic trials use physiological imaging to select the patient category that best matches the drug's presumed mode of action, rather than lumping together patients with entirely different pathophysiological patterns in so-called 'large trials', which have all failed so far.
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Abbreviations: CBF, cerebral blood flow; CBV, cerebral blood volume; DWI, diffusion-weighted imaging; HT, haemorrhagic transformation; MCA, middle cerebral artery; MMI, malignant middle cerebral artery infarction; MRI, magnetic resonance imaging; OEF, oxygen extraction fraction; PET, positron emission tomography; PWI, perfusion-weighted imaging; USPIO, ultrasmall superparamagnetic iron oxide

Introduction

Stroke is a leading cause of death and disability worldwide with far reaching consequences for the society (Feigin *et al*., 2003). The World Health Organization defines stroke as a rapidly developing focal (or global) brain dysfunction of vascular origin lasting more than 24 h, thus encompassing ischaemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage and cerebral venous sinus thrombosis (Brown *et al*., 2006).

Ischaemic stroke is by far the most common type of stroke, constituting around 80% of all strokes (Feigin *et al*., 2003), of which 60% are attributable to large-artery ischaemia. The current understanding of its pathophysiology has dramatically evolved over the past three decades, from early beginnings in animal studies through to the current wealth of information provided by various imaging techniques. This has transformed stroke care and ended decades of nihilism (Saver, 2006). Positron emission tomography (PET) has been particularly instrumental in these developments (Baron, 2005b) and continues to be the gold standard in stroke imaging. Other modalities such as computed tomography (CT), single photon emission CT and magnetic resonance imaging (MRI) have also assumed important roles both in the investigation of stroke pathophysiology and in applying its complex concepts to everyday clinical practice (Table 1). This article reviews the main pathophysiological models of ischaemic stroke and the role of imaging in formulating and implementing them and in the development of new therapeutic strategies.

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Table 1 Imaging modalities used to identify the ischaemic penumbra and core

Imaging modality	*Assessed parameters*	*Definition of penumbra*	*Advantages*	*Limitations*
CT				
Perfusion	CBF, CBV, MTT, TTP	Relative CBF $<66\%$[a] or MTT $>145\%$[a] and CBV $>2\,ml\,100\,g^{-1}$	Cheap, available, fast	Limited brain coverage, not sensitive in posterior circulation, no direct visualization of core
Xe	CBF	CBF 7–20 $ml\,100\,g^{-1}\,min^{-1}$	Quantitative	Technically complex, not validated, pharmacologic effects of Xe
MR				
DWI–PWI	CBF, CBV, MTT, TTP, ADC	Relative TTP (or MTT) delay $>4\,s$[a], relative CBF $<37\%$[a] & relative ADC above 50%[a]	Practical, fast, increasingly available, no radiation involved, directly visualizes severely ischaemic tissue	Uncertainties regarding validity of mismatch concept, sensitive to head motion
Spectroscopy	NAA, lactate	Elevated lactate and normal NAA	Biochemically characterizes tissue	Not widely available, not validated, poor resolution
PET				
Multi-tracer $^{15}O_2$	CBF, CBV, MTT, $CMRO_2$, OEF	CBF 7–22 $ml\,100\,g^{-1}\,min^{-1}$ and $CMRO_2>39\,\mu mol\,100\,g^{-1}\,min^{-1}$ and OEF $>70\%$	Quantitative, validated	Complex, time consuming, not widely available, expensive
^{11}C-FMZ ($+H_2^{15}O$)	Tracer binding	Relative binding ratio >3.4[b] and CBF $<14\,ml\,100\,g^{-1}\,min^{-1}$	Based on physiological neuronal integrity	*As above* + only suitable for grey matter, requires additional perfusion imaging
^{18}F-FMISO	Tracer uptake	Uptake ratio >1.3[a]	Produces a direct positive image of viable hypoxic tissue	*As above* + validation incomplete, long imaging time, not practical in acute setting
SPECT				
^{99m}Tc-HMPAO	CBF	Relative CBF 40–70%[a]	Cheap and relatively available	Thresholds still uncertain, limited spatial resolution

[a]Relative to mean contralateral hemisphere value.
[b]Relative to contralateral healthy white matter.
Abbreviations: ADC, apparent diffusion coefficient; CBF, cerebral blood flow; CBV, cerebral blood volume; $CMRO_2$, cerebral metabolic rate of oxygen; CT, computed tomography; DWI, diffusion-weighted imaging; FMISO, fluoromisonidazole; FMZ, flumazenil; HMPAO, hexamethylpropyleneamine oxime; MR, magnetic resonance; MTT, mean transit time; NAA, *N*-acetylaspartate; OEF, oxygen extraction fraction; PET, positron emission tomography; PWI, perfusion-weighted imaging; SPECT, single photon emission computed tomography; TTP, time to peak.

Basic concepts

Most experimental and clinical research have focused on proximal occlusion of the middle cerebral artery (MCA). Interruption of blood flow to the supplied basal ganglia, white matter and cortex causes a gradient of hypoperfusion to emerge (Figure 1), rather than complete and homogeneous ischaemia of the entire MCA territory (Astrup *et al.*, 1981). Regions suffering the most severe degrees of hypoperfusion rapidly progress to irreversible damage, representing the 'ischaemic core'. On multi-tracer ^{15}O PET, this tissue exhibits very low cerebral blood flow (CBF), cerebral blood volume (CBV) and metabolic rates of oxygen and glucose (Marchal *et al.*, 1999a). The remaining hypoperfused tissue exhibits impairment of the normal blood flow autoregulatory mechanisms and is pathophysiologically divided relative to a well-defined perfusion threshold into two compartments, namely, the 'penumbra' and 'oligaemia'. In the penumbra, oxygen metabolism is preserved relative to CBF, the oxygen extraction fraction (OEF) is elevated (severe 'misery perfusion') and the CBV is normal or elevated. Tissue within the penumbra is potentially salvageable, yet its extent decreases over time by gradual recruitment into the core and as such, represents a key target for therapeutic intervention (Baron *et al.*, 1995). This course of events varies from patient to patient, but most exhibit substantial volumes of penumbra for many hours (Baron, 1999) or exceptionally, days after stroke onset (Perez *et al.*, 2006). The oligaemic compartment, on the other hand, suffers a milder degree of hypoperfusion with normal oxygen consumption and elevated CBV and

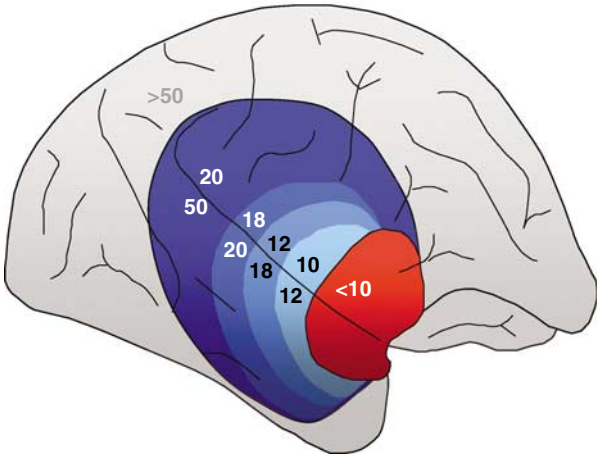

Figure 1 The spatial pattern of cerebral blood flow (CBF) reduction following middle cerebral artery (MCA) occlusion in the baboon brain, demonstrating a gradient from ischaemic core (red) through to penumbra and oligaemia (blue) to normally perfused cortex (grey). Values indicate approximate CBF in $ml\,100\,g^{-1}\,min^{-1}$.

OEF, and is not normally at risk of infarction. If the occlusion persists, however, secondary events such as systemic hypotension, intracranial hypertension or hyperglycaemia may topple this delicate balance and force the oligaemia into a penumbral state and eventually recruitment into the necrotic core.

The ischaemic core

The ischaemic core is by definition beyond therapeutic rescue. It is electrically silent and its volume is highly correlated to, and explains part of the severity of admission neurological deficit (Marchal *et al*., 1999a). On the cellular level, irreversible damage is heralded by depletion of energy metabolites and failure of the cell membrane to maintain its physiologic gradients with dramatic disruption of ion and water homoeostasis (Moustafa and Baron, 2007). This manifests as massive efflux of K^+ and reciprocal influx of Na^+, water and Ca^{++} with consequent anoxic depolarization (Branston *et al*., 1977; Harris *et al*., 1981) and eventually cell death. Additionally, large slow voltage shifts occur at the borders of the core and propagate as spreading depolarization waves that compromise tissue survival, including the penumbra (Selman *et al*., 2004).

In proximal MCA occlusion, the striato-capsular and opercular/insular regions are often the earliest to exhibit irreversible damage (Stoeckel *et al*., 2007). Subsequently, as the penumbra is recruited into the core, the latter progressively expands to other areas, including the cortical mantle. The maximum extent of the core will become the final infarct volume.

Haemodynamically, the core is defined as the tissue that exists below the perfusion threshold of infarction. This threshold was determined to be 5–8 $ml\,100\,g^{-1}\,min^{-1}$ within the first few hours after stroke onset, but rises progressively over time to reach the penumbra threshold (around 22 $ml\,100\,g^{-1}\,min^{-1}$) (Baron, 1999). Consequently, infarct expansion occurs earlier in tissue suffering more severe hypoperfusion. The corresponding infarction threshold defined by the cerebral metabolic rate of oxygen is around 39 $\mu mol\,100\,g^{-1}\,min^{-1}$ (Marchal *et al*., 1999a), and in contrast to CBF, does not appear to vary over time.

There are several other means of depicting the core (Table 1) that are gradually being validated against PET to allow application in everyday clinical practice. MRI diffusion-weighted imaging (DWI) is of particular importance, since it has exquisite sensitivity for acute ischaemia and can be positive within a few minutes from onset (Hjort *et al*., 2005). The DWI signal reflects restriction of the random motion of water in tissue and decline of its apparent diffusion coefficient and although the exact biological correlates of this phenomenon are incompletely understood, cytotoxic oedema and subsequent shrinkage of the extracellular space have been proposed (Nicoli *et al*., 2003). The volume of DWI abnormality correlates well with both admission and outcome neurological deficit as well as with final infarct volume (Baird *et al*., 2000). Yet studies in animals and humans document the potential reversibility of DWI lesions and normalization of apparent diffusion coefficient, thus arguing against its equivalence to the core (Li *et al*., 1999; Kidwell *et al*., 2000). Predictors of such normalization include thrombolytic therapy and recanalization, particularly within the 3-h time window (Fiehler *et al*., 2004), suggesting that the DWI lesion may include penumbral tissue. This has also recently been further confirmed in human PET-MR studies (Guadagno *et al*., 2006).

The core as a therapeutic target

Within the necrotic core, the vasculature may also be severely damaged, exposing it to the risk of undergoing haemorrhagic transformation (HT). This is especially the case with extensive infarction and with the use of thrombolytics, potentially causing further worsening of the clinical condition and outcome, and therefore outweighing the benefit of salvaging the ischaemic penumbra (Fiehler *et al*., 2005). Thus, although the core is essentially not recoverable, prediction and prevention of HT within it represent important therapeutic goals. For instance, new thrombolytic agents that do not interfere with endothelial function or induce matrix metalloproteinase dysregulation (Wang *et al*., 2004) might reduce the incidence of thrombolysis-associated haemorrhage. More generally, vascular protectants and agents that modulate matrix proteolysis may also be beneficial in preventing HT within the infarct (Lee *et al*., 2004).

In clinical trials, HT is often divided into grades based on radiologic appearance into small or confluent petechiae within the infarction (HI-1 and HI-2, respectively); parenchymal haematoma occupying $<30\%$ of the infarcted area, with a mild space-occupying effect (PH-1) and parenchymal haematoma in $>30\%$ of the infarcted area with a significant space-occupying effect (PH-2) (Larrue *et al*., 2001). These are further qualified as symptomatic and asymptomatic.

The pathophysiology of HT remains to be proven, but early reperfusion with injury to the microvasculature and disruption of the blood–brain barrier has been suggested. Indeed, some studies (Latour *et al*., 2004; Warach and Latour, 2004) have used delayed gadolinium enhancement of cerebrospinal fluid space on MR fluid-attenuated inversion recovery images as a marker of blood–brain barrier disruption and demonstrated its dependency on reperfusion and its association to HT and worse clinical outcomes. It is still not entirely clear, however, if these markers will be useful in clinical decision-making or in the development of new therapeutic strategies for HT.

A large parenchymal hypodensity on acute CT statistically predicts the risk of thrombolysis-associated haemorrhage, hence the widespread notion of withholding this treatment if hypodensity exceeds one-third of the MCA territory (Hacke *et al*., 1998). Studies using MRI have shown that areas of HT have significantly lower apparent diffusion coefficient, CBF and CBV than other areas with perfusion abnormality on initial imaging, and that reperfusion occurs in almost all cases showing HT (Selim *et al*., 2002; Alsop *et al*., 2005). Nonetheless, the results of these studies were not analysed separately for symptomatic and asymptomatic

grades of HT, and the relevance of these associations to clinical outcome is not identified. Lansberg *et al*. (2007a) investigated predictors of any haemorrhage causing symptomatic deterioration and found only a large DWI lesion to be an independent predictor in multivariate analysis. Thomalla *et al*. (2007) further argue that the HT is merely a frequent epiphenomenon related to reperfusion and that it is of little clinical consequence, distinguishing it from parenchymal haemorrhage related to IV thrombolytic therapy as a relevant target for investigation and prevention.

The ischaemic penumbra

The penumbra was originally described on electrophysiological basis as the tissue existing between the thresholds of electrical failure and ion pump failure (Astrup *et al*., 1981). Thereafter, a haemodynamic and metabolic approach based on multi-tracer ^{15}O PET has defined it as tissue that exists between the threshold of infarction and the penumbral threshold (Baron, 2001a). Operationally, penumbral tissue must satisfy criteria of being (i) functionally impaired viable hypoperfused tissue with undetermined fate that is at-risk of infarction if not salvaged; (ii) contributing to the clinical deficit and that (iii) its resolution is associated with proportional recovery of neurologic function.

Using multi-tracer PET, substantial volumes of cortical penumbra have been reported to decline over time, being present in over 50% of the patients studied within 9 h, and in about one-third of the patients studied between 5 and 18 h (Wise *et al*., 1983; Heiss, 1992; Marchal *et al*., 1996). This confirms that the temporal window of opportunity for therapy is protracted in some patients, but is rapidly shrinking in others, thus emphasizing the urgency of acute stroke management. Some patients develop an extensive necrotic infarct core within a few hours of stroke onset, whereas spontaneous reperfusion is seen in the remaining subgroup (Marchal *et al*., 1993).

The demise of the penumbra is signalled by a decline in cerebral metabolic rate of oxygen, with further decline or stabilization of the CBF (Wise *et al*., 1983; Heiss, 1992; Marchal *et al*., 1996) and a dramatic fall in the OEF, from initially very high to sometimes exceedingly low values heralding the exhaustion of the tissue's oxygen needs. Early reperfusion can reverse this grim outcome as shown in studies in baboons (Touzani *et al*., 1995, 1997) and humans (Heiss *et al*., 1998), reporting that large volumes of tissue with penumbral levels of CBF escape necrosis if arterial recanalization is achieved. Ample evidence from ^{15}O (Furlan *et al*., 1996; Heiss *et al*., 1998) and ^{18}F-fluoromisonidazole PET studies (Read *et al*., 2000; Markus *et al*., 2004) also indicates that survival of the penumbra has a definite and predictable benefit on subsequent neurological recovery in man. Less predictably, a better correlation also exists with 2-month recovery scores, suggesting that survival of the penumbra also influences late recovery, possibly through allowing subsequent peri-infarct neuronal reorganization (Furlan *et al*., 1996).

Similar findings have also been demonstrated using multimodal MRI combining DWI and perfusion-weighted imaging (PWI). Maps of CBF, CBV and mean transit time are generated by this latter technique, reflecting the perfusion status of brain tissue. Comparison of the perfusion deficit depicted on PWI with the DWI lesion (assumed to denote the core) thus yields either (i) a mismatch pattern (PWI > DWI); (ii) a matched lesion pattern (PWI = DWI) or (iii) a reperfusion pattern indicating recanalization (DWI > PWI). The mismatch pattern is taken to indicate the existence of salvageable at-risk tissue and is found in about 70% of patients with anterior-circulation stroke scanned within 6 h of onset (Barber *et al*., 1999). Its presence is strongly associated with proximal MCA occlusion and its resolution on reperfusion is associated with neurological recovery (Staroselskaya *et al*., 2001; Singer *et al*., 2004). Moreover, successful reperfusion prevents further expansion of the DWI lesion into the area of mismatch (Jansen *et al*., 1999). Nonetheless, as outlined earlier, uncertainties exist regarding the physiologic accuracy of the DWI lesion and corresponding uncertainties also exist regarding PWI, particularly in the selection of parameters for defining the tissue at risk and in the choice of arterial input function (Heiss *et al*., 2004; Rose *et al*., 2004; Sobesky *et al*., 2004). Thus, although the DWI–PWI mismatch concept is a very clinically useful tool, it may overestimate the penumbra by including oligaemic or even normally perfused but autoregulated tissue, that is not at-risk (Sobesky *et al*., 2005; Muir *et al*., 2006a). These questions also become particularly relevant when defining the management of matched DWI–PWI lesions, since response to recanalization largely depends on whether or not it includes penumbral tissue and therefore still likely to progress (Kane *et al*., 2007).

A simple alternative approach suggests inferring the presence of salvageable tissue by a mismatch between clinical stroke severity (National Institutes of Health Stroke Scale score $\geqslant$8) and the size of the DWI lesion. Studies during the past few years reported that this 'clinical–DWI mismatch' predicted infarct growth, neurologic deterioration (Davalos *et al*., 2004) and even the DWI–PWI mismatch (Prosser *et al*., 2005). A similar clinical–CT mismatch has also been proposed, yet recent evidence has cast doubt on the validity of both these approaches in reliably reflecting the presence of penumbra and in selecting suitable candidates for thrombolytic therapy (Kent *et al*., 2005; Lansberg *et al*., 2007b; Messe *et al*., 2007).

Other imaging strategies to detect the penumbra include single photon emission CT, xenon-CT and perfusion computed tomography, and their findings are generally consistent with other techniques (Muir *et al*., 2006a). Perfusion computed tomography has attracted special interest as it is similar in principle to MR PWI but has the practical advantage of being more widely available and cheaper than MR. Recent studies on perfusion computed tomography in acute stroke demonstrated that tissue with CBV $<2\,\mathrm{ml}\,100\,\mathrm{g}^{-1}$ represents the core, while a relative mean transit time above 145% of the normal hemisphere with CBV $>2\,\mathrm{ml}\,100\,\mathrm{g}^{-1}$ best outlines at-risk tissue (Wintermark *et al*., 2006). Perfusion computed tomography parameters correlate very well with MR DWI–PWI and accurately predict final infarct volume and clinical recovery, corroborating its potential utility in selecting patients for thrombolysis (East-

wood *et al*., 2003; Muir *et al*., 2006b; Wintermark *et al*., 2007) even when the time of onset is not clear (Hellier *et al*., 2006).

The penumbra as a therapeutic target

Reperfusion therapy in the 3-h window. In the mid-1990s, recombinant tissue plasminogen activator (alteplase) emerged as the first effective therapy aimed at rescuing at-risk tissue in the first hours of stroke. Large trials demonstrated that IV recombinant tissue plasminogen activator therapy affords at least a 30% increase in the likelihood of a good outcome when administered within 3 h, yet carries a 6–7% risk of symptomatic intracranial haemorrhage (Hacke *et al*., 2004). Those trials were based on plain (non-contrast) CT and thus did not distinguish stroke subtypes or objectively demonstrate the existence of penumbra, which suggests that more can be gained from thrombolysis by better selection of potential responders to treatment and exclusion of those who may not benefit or may be harmed. A multi-modal imaging approach based on MRI demonstration of DWI–PWI mismatch is thus increasingly being advocated as the investigation of choice in acute stroke though delay in treatment remains the primary concern (Kang *et al*., 2005). This is probably balanced by the added diagnostic accuracy and that shorter door-to-needle times can be achieved through omitting CT and tailoring MRI protocols to suit hyperacute stroke patients (U-King-Im *et al*., 2005).

Reperfusion beyond 3 h. The seminal recombinant tissue plasminogen activator trials undoubtedly revolutionized stroke therapy, yet they created an artificial cutoff at 3 h that may not apply to all patients (Baron *et al*., 1995). Indeed the pathophysiological model outlined earlier suggests that reperfusion can be beneficial beyond 3 h through salvage of the penumbra in appropriate patients. Extension of the therapeutic window is thus an attractive goal that is currently being pursued cautiously with the use of physiologic imaging.

The Diffusion and perfusion imaging Evaluation for Understanding Stroke Evolution study demonstrated a better clinical response among patients with small DWI lesion and substantial MR mismatch treated with alteplase between 3 and 6 h, than in other subgroups, including the matched DWI–PWI lesion, the small DWI and PWI lesion and the large DWI lesion subgroups (Albers *et al*., 2006). Thus, this important study highlighted the importance of considering not only the presence of DWI–PWI mismatch but also the size of the DWI lesion in the decision-making process. The ongoing EPITHET trial further addresses this question by randomizing patients to alteplase or placebo 3–6 h after stroke onset regardless of baseline MRI findings, testing the hypothesis that in retrospective analysis patients with mismatch will have derived greater benefit than those without (Butcher *et al*., 2005).

Studies comparing MRI-based alteplase treatment within 3–6 h to conventional CT-based treatment within 3 h have demonstrated similar recanalization rates and functional outcomes (Rother *et al*., 2002; Ribo *et al*., 2005). Moreover, MRI-based treatment in the 0–6 h time frame also shows similar or superior safety and efficacy to CT-based treatment within 3 h, when compared directly (Kohrmann *et al*., 2006) or to data from meta-analyses (Thomalla *et al*., 2006). Preliminary findings from pooling of results from 1210 patients further strengthen these conclusions (Schellinger *et al*., 2007).

MR-based selection has also been used in studies testing the new thrombolytic agent desmoteplase (Hacke *et al*., 2005), where the presence of MR DWI–PWI mismatch of 20% or higher was used to select patients for thrombolysis in the 3–9 h window. A more favourable clinical outcome was demonstrated in patients who experienced reperfusion than in those who did not (52.5 vs 24.6%), and the treatment effect was independent of the duration from onset to treatment. Nonetheless, a further phase 3 trial on desmoteplase (The Desmoteplase in Acute Ischemic Stroke Trial II) (Hacke and Furlan, 2007) has failed to reproduce the same results, which arguably casts doubt on the use of the mismatch model to select patients for treatment. However, the negative results also suggest that the drug's efficacy may have been overestimated in previous studies or that this treatment window is too late. The small number of participants in each arm of the trial ($n \sim 60$ in each group) may have also contributed to the lack of a detectable benefit over placebo. Pro-urokinase is another thrombolytic agent that has been investigated for use in acute stroke. The PROACT II study used catheter angiography and plain CT to select patients with MCA occlusion for intra-arterial pro-urokinase treatment up to 6 h from onset (Furlan *et al*., 1999). The findings were strongly in favour of a beneficial effect on clinical outcome, and subsequently it was shown that detailed analysis of the patients' CT scans (Hill *et al*., 2003) may further improve patient selection for this treatment. Other thrombolytic agents (such as tenecteplase) and other intra-arterial reperfusion techniques are also under investigation in acute stroke and imaging is increasingly being used to monitor their therapeutic effects (Molina and Saver, 2005).

Neuroprotection. When tested in humans, neuroprotective agents designed to limit the demise of at-risk tissue have consistently failed to produce the effects observed in animal studies (Savitz and Fisher, 2007; Shuaib *et al*., 2007). These agents targeted critical interlinked events that occur in ischaemic tissue before or after reperfusion ending in necrotic cell death (the 'ischaemic cascade'). Their failure is variously attributed, among many possibilities, to inadequate preclinical data or therapeutic targets, and the choice of ineffective compounds (Green, 2002). Importantly, physiologic imaging has only been employed in very few of these trials (for example, Warach *et al*., 2000), so the grouping together of patient populations who may not even have the substrates targeted by such therapies (for example, penumbra) is another likely factor for the failure of translation to humans.

A further reason that is proposed is that most neuroprotective drugs were designed to specifically reduce damage to the cortical grey matter rather than the white matter (Dewar *et al*., 1999). Several studies using PET and MRI have demonstrated that substantial volumes of potentially salvageable tissue existed in white matter several hours after

onset of stroke and that it is at least as resistant to ischaemia as grey matter (Falcao *et al*., 2004; Koga *et al*., 2005; Simon *et al*., 2005). Furthermore, an MRI study by Bristow *et al*. (2005) quantitatively demonstrated that grey matter was at risk of infarction at higher CBF values and at shorter mean transit time delays than white matter. These findings thus emphasize the necessity of devising approaches that target not only grey matter but also white matter ischaemia when considering novel strategies to neuroprotection.

Another potential strategy for neuroprotection is prehospital administration of treatment that can essentially 'buy time' until imaging can be undertaken and definitive therapy instituted. Such a neuroprotectant clearly has to be safe and tolerable in both ischaemic and haemorrhagic strokes, and should be simple to administer by ambulance personnel. Magnesium sulphate is one such candidate and is currently being tested within 2 h of stroke onset in a large clinical trial (Saver *et al*., 2004).

On a more general standpoint, the failure of clinical trials on agents targeting solely the neurons together with the evolving understanding of the key roles played by other cell types, support the concept of an integrative approach to neuroprotection that replaces the prevailing neurocentric paradigm (Lee *et al*., 2004). This approach addresses the various interacting components that make up the neurovascular unit, namely, the neuronal tissue, the glial tissue and supporting matrix, and the cerebral vasculature (Singhal *et al*., 2005b).

Oxygen therapy. The scarcity of blood supply within the penumbra relative to its oxygen needs means that it is hypoxic and its function is reversibly affected by the reduction in tissue partial pressure of oxygen rather than hypoperfusion *per se*. This carries two main implications: (i) that increasing the partial pressure of oxygen in inspired air may be an effective therapeutic option and (ii) that mapping of the tissue partial pressure of oxygen in the brain would provide a direct way of depicting salvageable penumbral tissue, either alone or in conjunction with CBF measurements. One such approach is the use of the PET tracer ^{18}F-fluoromisonidazole and other nitroimidazoles. Tissue identified using this technique shares the operational criteria of the ischaemic penumbra (*see above*) and its fate correlates to clinical outcome up to 48 h from stroke onset (Markus *et al*., 2004). Nonetheless, among other drawbacks, validation is not yet complete and the long scanning time required for this technique still precludes its use outside the research setting.

Studies on oxygen therapy initially focused on administration of hyperbaric oxygen, but this later became replaced by normobaric oxygen owing to its wider availability, ease of administration and safety (Singhal, 2007). Almost all experimental studies on normobaric oxygen therapy showed significant reduction of infarct size, and recently further demonstrated that it improves CBF and oxygenation, at least in part, by inhibiting peri-infarct depolarization waves and hence reducing oxygen demand (Shin *et al*., 2007).

Promising results also exist from small clinical trials. In one pilot study (Singhal *et al*., 2005a), MRI DWI–PWI mismatch was used to select acute stroke patients to receive either 100% oxygen or room air for 8 h. Oxygen-treated patients improved clinically during therapy and at 24 h, with smaller MR DWI lesions than in control subjects. Moreover, oxygen therapy was associated with an increase in relative CBF and CBV within the perfusion (mean transit time) abnormality, consistent with earlier observations of a vasodilatory response to hyperoxia in ischaemic brain tissue rather than the vasoconstriction induced in normal brain tissue (Nakajima *et al*., 1983). Another small trial (Chiu *et al*., 2006) did not use an imaging end point but showed that 40% venturi mask oxygen therapy reduced mortality and morbidity in patients with large stroke. Larger trials using similar methodologies and physiological imaging are awaited.

The oligaemia

Oligaemic tissue exists at a CBF range above the penumbra threshold and though it shows a mild degree of misery perfusion (high OEF) on PET, it is not normally at risk of infarction (Furlan *et al*., 1996). Thus, misery perfusion should not be equated with penumbra in acute stroke.

Cellular changes that occur in the oligaemic blood flow range in acute stroke are limited to differential induction and inhibition of protein synthesis. This likely represents the activation of a cellular stress response in which heat-shock proteins, unfolded proteins, endoplasmic reticulum kinases, caspases and many others are involved (DeGracia, 2004). Prolonged persistence of cellular stress responses initiates apoptotic programmed cell death and hence may explain the selective loss of neurons in areas remote from the penumbra and core of cerebral ischaemia (Paschen and Mengesdorf, 2005). Current therapeutic interventions do not specifically target the oligaemic compartment, apart from prevention of secondary insults such as systemic hypotension and hyperglycaemia which may threaten the oligaemia and incorporate it into the at-risk compartment (Baron, 2001b).

Secondary events and contributors

Imaging has been used to identify secondary contributors to ischaemic injury and investigate their influence on tissue outcome. Multi-modal MRI constitutes the main tool in these studies because of its relative availability and tolerability in the acute setting.

Hyperglycaemia

Parsons *et al*. (2002) explored the association of hyperglycaemia with the fate of at-risk hypoperfused tissue. They showed that acute hyperglycaemia was independently correlated with reduced tissue survival and that higher blood glucose levels were also strongly correlated with larger final infarct sizes and worse functional outcomes. Furthermore, using proton MR spectroscopy, they demonstrated that higher acute blood glucose in patients with DWI–PWI mismatch was associated with greater lactate production,

which, in turn, was independently associated with the reduced salvage of mismatched tissue. Baird *et al*. (2003) similarly demonstrated that high acute blood glucose levels were strongly correlated with infarct expansion on serial MRI and with worsening of functional outcomes. It is, however, still not certain if hyperglycaemia is in itself detrimental in acute stroke or that it is rather a manifestation of a more fundamental and injurious process such as sympathetic activation or hypercortisolism. This is emphasized by the failure of a large trial of intensive insulin therapy to detect any functional benefit despite achieving sustained euglycaemia (Gray *et al*., 2007). Yet this trial had a number of shortcomings. First, physiological imaging was not employed in selecting patients; the trial was prematurely terminated due to slow recruitment and a wide range of plasma glucose levels were considered abnormal (6–16 mM) and actively treated for a target capillary glucose of 4–7 mM. This may not have been an adequate goal, and given that glucose–potassium–insulin infusions also effected a significant drop in systemic blood pressure in the treatment group, the results ought not be considered definitive.

Haematocrit

Elevated blood haematocrit has been shown to associate with infarct expansion and reduced penumbral tissue salvage (Allport *et al*., 2005). These effects are probably mediated by increased blood viscosity and impairment of capillary flow. Haemodilution therapy was previously a popular approach in addressing this problem, but enthusiasm has faded following the failure of several trials to demonstrate substantial clinical benefit (Asplund, 1989).

Systemic blood pressure

Demonstration of high OEF or DWI–PWI mismatch in the setting of acute stroke implies that autoregulation of CBF is impaired in the affected territory. Thus, any lowering of the systemic arterial pressure is likely to further reduce the cerebral perfusion pressure and in turn the CBF in the affected tissue, which can be harmful for the penumbra as well as the oligaemia. Accordingly, blood pressure reductions in acute ischaemic stroke have frequently been associated with worse outcome (Ahmed *et al*., 2000), especially with iatrogenic lowering of reactive hypertension. Conversely, observing hyperperfusion, particularly if early oedema is demonstrated by CT or MRI, may provide rationale for treating hypertension as it is suggested that hyperperfusion in necrotic tissue may promote the development of malignant brain swelling (Marchal *et al*., 1999b). Several trials are currently underway to address these questions and assess the optimum management of blood pressure in the acute stage, including the UK-based Controlling Hypertension and Hypotension Immediately Post-Stroke Trial (Potter *et al*., 2005); The Continue or Stop post-Stroke Antihypertensives Collaborative Study (COSSACS) (2005) and the international multi-centre 'Efficacy of Nitric Oxide in Stroke' study. Another large international study (INTERACT) is exploring the optimal approach to managing blood pressure specifically after intra-cerebral haemorrhage.

Vasogenic oedema

Vasogenic oedema usually develops in the first 2–3 days following the onset of stroke causing swelling of the brain tissue. Oedema is usually of modest clinical impact unless associated with a large rapidly developing space-occupying MCA infarction. This is known as malignant MCA infarction (MMI) owing to its very poor prognosis under standard therapy, with a case-fatality rate approaching 80% (Vahedi *et al*., 2007). The reasons behind the development of MMI are not clearly understood, but some evidence points to factors beyond the size of infarction, such as inflammation and blood–brain barrier breakdown, as instrumental mechanisms (Serena *et al*., 2005). Substantial vasogenic oedema increases local tissue pressure and thus reduces the effective perfusion pressure. This, in turn, can lead the penumbra to progress to infarction and hence lead to further infarct expansion with development of more oedema and a vicious cycle ensues.

Predicting the development of MMI as early as possible is important to allow timely institution of therapy. Imaging-based predictors include occlusion of the proximal MCA, carotid T occlusion, involvement of both the superficial and deep MCA territories, inadequate circle of Willis and involvement of other vascular territories (Jaramillo *et al*., 2006). PET and single photon emission CT allow accurate prediction of MMI (Marchal *et al*., 1995; Berrouschot *et al*., 1998), but the more clinically available DWI MRI is also of considerable potential. A large DWI lesion volume (>145 ml within 14 h or >82 ml within 6 h) reportedly predicts MMI with 100% sensitivity and 94% specificity (Oppenheim *et al*., 2000; Thomalla *et al*., 2003).

Anecdotal clinical reports of decompressive surgery for MMI prompted experimental studies that demonstrated a beneficial effect on infarct size and outcome (Forsting *et al*., 1995). Eventually, several clinical trials have shown that decompressive surgery, in the form of wide hemicraniectomy and duraplasty performed within 48 h of stroke onset reduces mortality by an absolute 50% and improves functional outcome in the survivors, although less impressively (Vahedi *et al*., 2007). Early decompression prevents life-threatening brain herniation and probably also reduces the detrimental effects of raised intracranial pressure on tissue perfusion pressure. Induction of moderate hypothermia (around 33 °C) has also been used in the treatment of MMI and some small open studies showed a beneficial effect on clinical outcome (Schwab *et al*., 2001; De Georgia *et al*., 2004), though carrying the risks of pneumonia and rebound increase in intracranial pressure on re-warming. On the other hand, osmotically active agents (for example, mannitol) and steroids offer little benefit in limiting the progression of MMI, and hence devising novel pharmacological approaches to brain oedema remains an area of potential future development.

Inflammation

Inflammation is thought to contribute to the pathophysiology of neuronal cell death by several mechanisms, including apoptosis (Price *et al*., 2003). Within minutes of ischaemia, proinflammatory genes are upregulated and adhesion mole-

cules are expressed on the vascular endothelium. Neutrophils then migrate from the blood into the brain parenchyma within hours after reperfusion (Emerich *et al*., 2002), followed by macrophages and monocytes within a few days. The vast majority of macrophages in the infarct area appear to be derived from local microglia that are activated before macrophage infiltration from the blood (Schilling *et al*., 2003), although the temporal pattern is not entirely clear. Animal studies suggest that microglial activation also extends beyond the core and could contribute to peri-infarct neuronal death (Mabuchi *et al*., 2000). Conversely, some evidence also exists for a beneficial or protective role for inflammatory cell recruitment and activation in the ischaemic process (Danton and Dietrich, 2003), including promotion of plasticity and modulation of neurotrophic factors (Lalancette-Hebert *et al*., 2007). Consequently, it is still unclear whether or not strategies to nonspecifically downregulate this response would in fact improve outcome and limit neuronal damage.

The advances in *in vivo* imaging of inflammation in stroke have expanded the investigation of this phenomenon, particularly in the clinical setting. The PET tracer ^{11}C-PK11195 binds to the peripheral benzodiazepine receptor, which is abundant on brain-derived activated microglia, and thus has been employed in experimental and clinical studies addressing ischaemia-related inflammation. In general, the results of these studies agree that microglial activation becomes significant after a few days of stroke and persists for over 30 days, with a peak around 2 weeks from stroke onset (Sette *et al*., 1993; Gerhard *et al*., 2005; Price *et al*., 2006). Notably, the spatial distribution of PK11195 binding evolves to include not only the core but also the rescued penumbra where inflammation may be secondary (or contributes) to selective neuronal damage (Baron, 2005a). Increased PK11195 binding may also be seen in brain regions distant from the infarct, including the contralateral hemisphere, possibly representing remote Wallerian degeneration (Pappata *et al*., 2000). These findings suggest that potential targets for therapy may still exist for weeks after stroke onset. A paramagnetic MRI contrast agent (ultrasmall superparamagnetic iron oxide), which is primarily taken up by blood-derived macrophages, has also been used to demonstrate parenchymal macrophage infiltration in animals and humans following ischaemic stroke (Kleinschnitz *et al*., 2003; Saleh *et al*., 2004; Wiart *et al*., 2007). The effect was prominent in the second week after stroke and was independent of blood–brain barrier disruption and lesion size (Nighoghossian *et al*., 2007). PK11195 and ultrasmall superparamagnetic iron oxide thus target two potentially overlapping components of the brain inflammatory response and further work using these two attractive techniques can be expected to expand the understanding of inflammation in stroke and guide therapeutic innovation.

Conclusions

Physiological imaging has elucidated many of the fundamental processes of ischaemic brain injury and demonstrated the substantial heterogeneity among individual stroke patients. The prolonged persistence of salvageable penumbral tissue has been established, and several other potential targets for intervention are gradually emerging. In future trials of therapeutic agents, the use of physiological imaging to select the patient category that best matches the drug's presumed mode of action is recommended, rather than lumping together patients with entirely different pathophysiological patterns in the so-called 'large trials', which have all failed so far. This approach promises to bring about further significant advances in the treatment of ischaemic stroke. Furthermore, imaging has also highlighted the important roles of the brain vasculature, glia and supporting matrix in stroke, all of which represent potential targets for therapy beyond neuroprotection *per se*.

Conflict of interest

The authors state no conflict of interest.

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