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Role of cerebral glutamate in post-stroke epileptogenesis

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ABSTRACT

Stroke is one of the most important causes of acquired epilepsy in the adult population. While factors such as cortical involvement and haemorrhage have been associated with increased seizure risk, the mechanisms underlying the development of epilepsy after stroke remain unclear. One hypothesised mechanism is an excitotoxic effect of abnormal glutamate release following a stroke. Cerebral extracellular glutamate levels are known to rise in the setting of acute stroke, and numerous studies have implicated glutamate in the pathogenesis of seizures and epilepsy, both through direct measurement of glutamate from the epileptic brain and by analysis of receptors and transporters central to glutamate homeostasis. While experimental evidence suggests the cellular injury induced by glutamate exposure may lead to development of an epileptic phenotype, there is little direct data linking the rise in glutamate during stroke with the later development of epilepsy.

Clinical research in this field has been hampered by the lack of non-invasive methods to measure cerebral glutamate. However, with the increasing availability of 7T MRI technology, Magnetic Resonance Spectroscopy is able to better resolve glutamate from other chemical species at this field strength, and Glutamate Chemical Exchange Saturation Transfer (*GluCEST*) imaging has been applied to localise epileptic foci in non-lesional focal epilepsy.

This review outlines the evidence implicating a pivotal role for cerebral glutamate in the development of post-stroke epilepsy, and exploring the role of MRI in studying glutamate as a biomarker and therefore its suitability as a molecular target for anti-epileptogenic therapies. We hypothesise that the rise in glutamate levels in the setting of acute stroke is a clinically relevant biomarker for the development of post-stroke epilepsy.

1. Introduction

1.1. Post-stroke epilepsy

Stroke is one of the most important causes of acquired epilepsy in the adult population (Hauser et al., 1993). Management of post-stroke epilepsy is of great clinical significance as patients with seizures after stroke have higher mortality and disability than those without seizures (Xu et al., 2016). Furthermore, epilepsy impairs long-term functional outcomes in those who have suffered a stroke or transient ischaemic attack (Arntz et al., 2013).

Several studies have investigated the development of seizures and epilepsy following stroke. The reported incidence varies significantly between studies; this likely reflects differences in definition, variable inclusion of patients with haemorrhagic stroke or subarachnoid haemorrhage, and significant clinical heterogeneity. Furthermore, most studies include both early and late seizures, with various definitions of early seizures ranging from seizures at the time of stroke onset to 2 weeks afterward. A cohort study of patients with ischemic stroke from our institution found an incidence of early onset seizures of 5% and late onset seizures of 10.1% over two years in 159 patients treated with IV rtPA, and 5.8% and 8% in 138 non rtPA treated patients (Tan et al., 2012). A systematic review and pooled analysis of 102,008 patients (Zou et al., 2015) showed an overall incidence of post-stroke seizures of 7%, with those with haemorrhagic stroke or cortical involvement at higher risk. There is evidence that stroke associated with venous sinus thrombosis also confers a higher risk of seizures (Benbir et al., 2006).

1.2. Revised definition of post-stroke epilepsy

According to the International League Against Epilepsy, acute symptomatic seizures are defined as those seizures occurring in close

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temporal association with a brain insult. This is reflected in the differentiation between "early" and "late" post-stroke seizures, with the latter defined as occurring more than 7 days after the stroke (Beghi et al., 2010). While epilepsy has historically been defined as two or more unprovoked seizures, the revised ILAE definition of epilepsy from 2014 included individuals with one unprovoked seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures, occurring over the next 10 years (Fisher et al., 2014). Hence according to the new definition an individual experiencing one post-stroke seizure at least one month after stroke (or two seizures at least 7 days after stroke) can be diagnosed with epilepsy. This is consistent with previous observational studies that show most patients with one late post-stroke seizure will go on to develop recurrent seizures (De Reuck et al., 2008).

1.3. Risk factors for post-stroke epilepsy

Galovic and colleagues recently proposed a multivariable prediction model for the development of late seizures after ischaemic stroke, drawing on five identified predictive factors of stroke severity, large artery atherosclerotic aetiology, early seizures, cortical involvement and middle cerebral artery territory involvement (Galovic et al., 2018). Based on this algorithm, those at highest risk were determined to have a post-stroke epilepsy risk of 83% within five years.

Our research group has reported several risk factors for the development of post-ischaemic stroke seizures, including cortical involvement (Koome et al., 2016), a low Alberta Stroke Program Early CT Score (ASPECTS) score (Chen et al., 2017) and haemorrhagic transformation (Thevathasan et al., 2017). We have also demonstrated that implementation of a code stroke system reduces the risk of post-stroke epilepsy (Chen et al., 2018).

1.4. Treatment and prevention of post-stroke epilepsy

While anti-epileptic drugs form the first line for management of post-stroke epilepsy the optimal timing and choice of drug have not been established. There have not been any randomised controlled trials comparing an anti-epileptic drug with placebo for the secondary prevention of post-stroke seizures (i.e. after the occurrence of one or more seizures). A recent Cochrane review commented on three randomised trials comparing different secondary prevention therapies (Sykes et al., 2014), only one of which examined a cohort in which stroke was the only aetiology for the seizures (Gilad et al., 2007). Across these studies lamotrigine was shown to be both efficacious and to have a superior tolerability profile to the other agents. Levetiracetam may also be a safe and effective option in elderly patients (Consoli et al., 2012). Post-stroke seizures generally respond well to anti-epileptic drug treatment: Gilad and coworkers observed seizure freedom in 37/64 patients over a 12 month follow-up period (Gilad et al., 2007).

Two studies have examined anti-epileptic drugs for the primary prevention of seizures after stroke (i.e. anti-epileptogenic therapy). Van Tuijl et al. (2011) studied whether the short-term (14 week) administration of levetiracetam would prevent the development of epilepsy after stroke. This study was prematurely terminated due to slow recruitment rates. A randomised controlled trial with a placebo group has examined the role of short-term antiepileptic therapy to prevent late seizures after haemorrhagic stroke (Gilad et al., 2011); the authors found that treatment with sodium valproate for one month did not affect seizure risk. Our group has reported that patients developing haemorrhagic transformation after receiving reperfusion therapy for acute ischaemic stroke are at higher risk of epilepsy and may benefit from longer follow-up (Naylor et al., 2018).

2. Glutamate

For an effective anti-epileptogenic therapy to be developed, consideration needs to be given to the identification of biomarkers for patients at most risk of developing post-stroke seizures. While various studies have looked at the peri-stroke exposome and association with seizures, including relevant comorbidities, metabolic disturbance and concomitant pharmacotherapy, no other biomarkers have been identified (Pitkänen et al., 2016).

Cerebral glutamate may be a biomarker of post-stroke seizures. Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and plays a key role in normal brain function. Most brain glutamate is present intracellularly however as glutamate receptors are located on the cell surface its extracellular concentration determines the extent of stimulation (Danbolt, 2001). Glutamate-mediated excitotoxicity refers to the process by which excessive stimulation of glutamate receptors induces neuronal cell death, a process implicated in several neurological disorders including stroke, epilepsy, brain trauma and chronic neurodegenerative disorders (Wang and Qin, 2010). Overstimulation of glutamate receptors induces cell death by multiple effects including impairment of intracellular calcium homeostasis, activation of proteases and kinases, increased nitric oxide and expression of pro-death transcription factors (Wang and Qin, 2010).

Activation of glutamate receptors has been shown to contribute to ischaemic cell death in stroke by impairing ion homeostasis with a resultant increase in intracellular calcium concentration (Dirnagl et al., 1999). Tissue oedema arises through influx of sodium and chloride ions and subsequently water, impairing perfusion of regions surrounding the ischaemic core.

It is well documented however that changes in the strength of excitatory synapses play an important role in synaptic plasticity (Derkach et al., 2007) and it is postulated that glutamate receptor signalling may therefore play a different role in the recovery from stroke to during the phase of acute ischaemia (Carmichael, 2012). Among the evidence supportive of this theory is the observation that motor recovery is enhanced by increased α–amino-3-hydroxy-5-methyld-4-isoxazolepropionic acid (AMPA) receptor signalling (Clarkson et al., 2011). Chronic treatment with the N-methyl D-aspartate (NMDA) receptor antagonist memantine, however, improved recovery from stroke in an animal model (López-Valdés et al., 2014).

2.1. Glutamate and stroke

In animal models, acute elevation of glutamate levels have been observed after stroke (Takagi et al., 1993) with the highest elevations seen in those with the largest infarctions. Other studies however have demonstrated a decrease in glutamate levels, postulated due to a combination of decreased glutamate synthesis and loss of brain glutamate via cerebrospinal fluid and the systemic circulation (Håberg et al., 2001). In human studies, elevated intra-cerebral glutamate levels, as measured from cerebrospinal fluid, have been demonstrated in acute stroke (Castillo et al., 1996) with glutamate levels in both CSF and plasma correlated with infarction lesion and severity of neurological deficit (see Fig. 1). Glutamate elevation has also been implicated in the pathogenesis of haemorrhagic stroke, however this may involve a different mechanism through Src kinase activation and phosphorylation of glutamate receptors (Keep et al., 2012).

2.2. Glutamate and epilepsy

The glutamate/GABA hypothesis of epilepsy is one of the extensively researched theories of epileptogenesis. Several aspects of the glutamate pathway have been studied, including neuro- and gliotransmission of glutamate, sensing transmitter molecules and the role of glutamate and GABA receptors (DiNuzzo et al., 2014).

Changes in glutamate levels in the human epileptic brain are well established. During and Spencer (1993) measured glutamate and GABA concentrations by microdialysis in six patients with temporal lobe

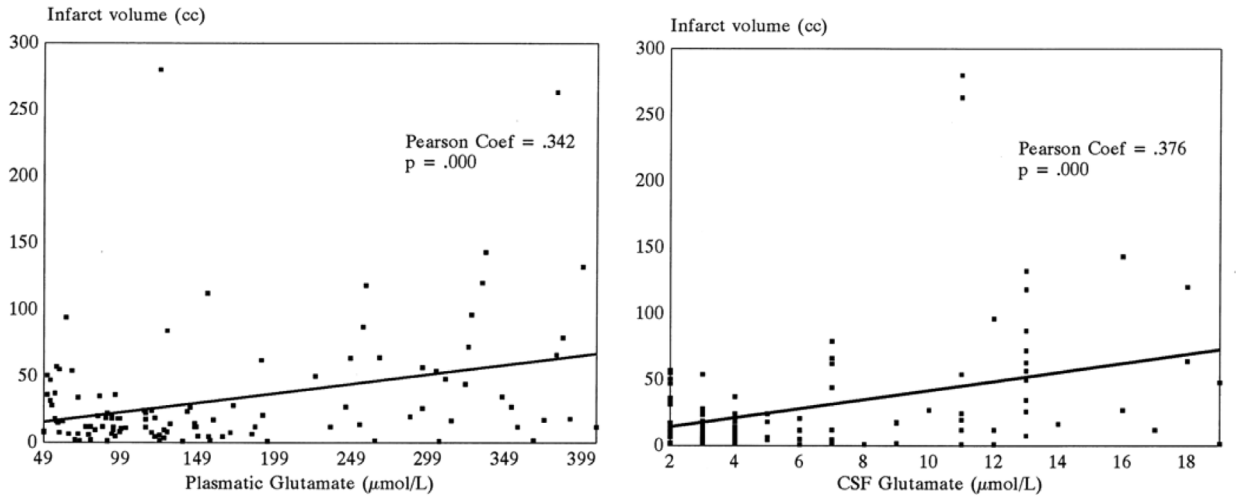

Fig. 1. The relationship between infarct volume and glutamate levels, from Castillo et al. (1996).

epilepsy undergoing epilepsy surgery, identifying high levels of glutamate in the epileptogenic hippocampus both prior to and during seizures, therefore concluding that the rise in extracellular glutamate may precipitate seizures. Cavus et al. performed microdialysis studies on 79 patients at the Yale epilepsy surgery program (Çavuş et al., 2016). The authors demonstrated elevated glutamate levels in epileptogenic, non-localised and lesional cortical sites when compared to non-epileptogenic cortex. They also found elevated glutamate levels in epileptogenic compared with non-epileptogenic hippocampus. Our group have reported elevated glutamate levels in the tumours and surrounding peri-tumoural cortex in resected tissue from patients with brain tumour-related epilepsy compared to those with histologically similar brain tumours without epilepsy (Neal et al., 2016; Yuen et al., 2012).

There are other lines of evidence implicating glutamate in the pathogenesis of epilepsy and epileptogenesis. Changes in excitatory and inhibitory glutamate transporters, for example, have been observed in epileptogenic brain regions (Rakhade and Loeb, 2008), and experimentally altering the function of glutamate receptor proteins can induce epilepsy syndromes (Meldrum et al., 1999).

2.3. Modulation of glutamate levels as a therapeutic target

The experience with glutamate receptor antagonists in stroke has however been a disappointing one. For example, despite observed neuroprotective properties in animal studies, clinical trials of the NMDA receptor antagonist selfotel were stopped prematurely due to a trend toward increased mortality in the treatment group (Davis et al., 2000) with similar failures observed in the setting of traumatic brain injury (Morris et al., 1999). A trial of the AMPA receptor antagonist ZK200775 was also stopped due to transient neurological deterioration in the treatment group (Elting et al., 2002). While some have cited pharmacokinetic factors and poor trial design as possible explanations for the failure, it has also been postulated that synaptic activity mediated by NMDA receptors may promote survival of neurons in this setting (Ikonomidou and Turski, 2002).

Several studies have looked at the role of modulators of the glutamate pathway in both treating epilepsy and preventing epileptogenesis. A well-known example is the non-competitive, selective AMPA receptor antagonist perampanel, approved as adjunctive treatment for refractory focal and generalised seizures based on demonstrated efficacy in several multi-centre randomised trials of patients with uncontrolled focal and generalised seizures (French et al., 2015). While it is not known if perampanel has anti-epileptogenic properties in humans, in animal studies it has been shown to modulate the development of epilepsy induced by kindling techniques (Dupuis et al., 2017). It has also been shown to improve cognitive outcome after middle cerebral artery occlusion in rats although the association with development of seizures was not specifically studied (Nakajima et al., 2018).

Numerous other studies have demonstrated attenuation of epileptogenesis through modulation of both ionotropic and metabotropic glutamate receptors. Administration of pre-synaptic metabotropic glutamate agonists has been shown to block the development of epileptogenesis, correlated with a decrease in glutamate release from cortical synaptosomes (Attwell et al., 1995) as has antagonism of excitatory metabotropic receptors prior to administration of pilocarpine (Jesse et al., 2008). NMDA receptor antagonists have been shown to suppress the development of epilepsy from amygdala kindling (Gilbert, 1988) and antagonism of both NMDA and AMPA receptors concurrently has been shown to attenuate kindling while slowing the development of pathological changes (Schidlitzki et al., 2017).

Several studies have induced experimental stroke by means of prothrombosis or middle cerebral artery occlusion although the rate of development of seizures is variable and differs depending on the type of stroke induction (Pitkänen et al., 2016). In one study, the administration of the pyrrolidone derivative nefiracetam reduced the frequency and duration of non-convulsive seizures when administered 15 min after middle cerebral artery occlusion (Lu et al., 2013) and also protected against neuronal cell death induced by glutamate in the in vitro setting. The non-competitive AMPA receptor antagonist EGIS-8332 demonstrated both anticonvulsant properties as well as neuroprotective effects against ischaemic injury induced by several mechanisms (Gigler et al., 2007).

2.4. Modelling post-stroke epileptogenesis: the role of glutamate

Despite the evidence implicating glutamate in both stroke and epilepsy, there is little data examining the relationship between the rise in glutamate during acute stroke with the risk of developing subsequent seizures. Xie et al. (2016) demonstrated elevated plasma glutamate levels and decreased calcium levels in stroke patients with early seizures (within 72 h) compared with those without seizures although the effect on development of late seizures remains unclear.

In-vitro methods have also been employed. In one such study, injury was induced by exposure of cultured neurons to glutamate, a process analogous to glutamate-mediated cell death in stroke, with Spontaneous Recurrent Epileptiform Discharges (SREDs) observed in surviving neurons. The same authors have shown that glutamate injury-induced epileptogenesis is calcium-dependent and is blocked by administration of a noncompetitive NMDA receptor antagonist (DeLorenzo et al., 2007). Some authors have contended however that

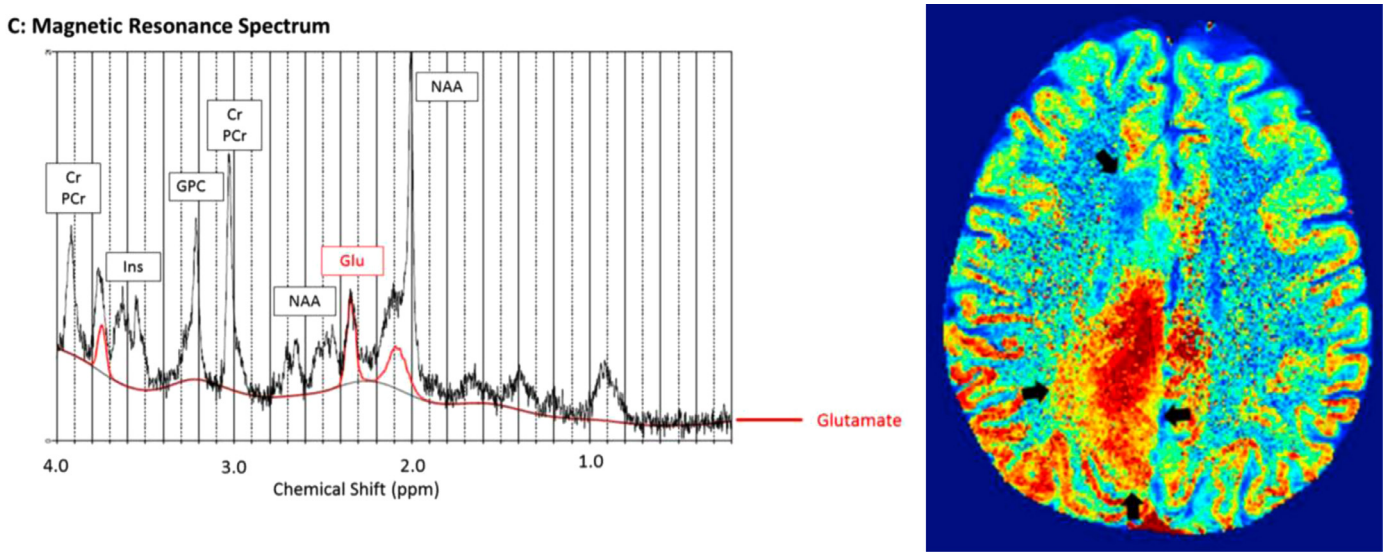

Fig. 2. Magnetic Resonance Spectrum at 7T (left). Axial GluCEST image (right) demonstrating increased GluCEST contrast in a glioma patient. From Neal et al. (2019).

exposure to glutamate in vitro is not analogous to in-vivo ischaemic injury as the metabolic and biochemical responses are different (Di Renzo et al., 2009).

2.5. The role of imaging

One of the inherent challenges in searching for biomarkers of post-stroke seizures is that quantification of intracerebral metabolites has relied on direct measurement from brain tissue or cerebrospinal fluid. The advent of 7T MRI technology represents an opportunity for imaging-based quantification of intracerebral metabolites in vivo. Magnetic Resonance Spectroscopy (MRS) studies of stroke at lower field strengths have demonstrated prolonged elevations in lactate and reduced N-acetylasparatate (NAA), thought to be a surrogate marker of cell survival or loss (Graham et al., 1993). While glutamate is difficult to separate from glutamine and GABA at 1.5 and 3T, Bivard et al. (2014) observed increased glutamate as part of a metabolic signature including elevated lactate and NAA in hyper-perfused brain tissue after reperfusion therapy. Interestingly this pattern was associated with better clinical outcomes after reperfusion.

MRS has also been used extensively in the study of epilepsy. A systematic review of MRS studies in juvenile myoclonic epilepsy identified metabolic changes across a range of brain regions including the thalamus, frontal lobe insula and striatum (Zhang et al., 2016). Regional increases in the glutamate-glutamine complex (Glx) may be the result of hyperexcitability (Lin et al., 2009).

Several studies of focal epilepsy have showed reduced NAA ipsilateral to seizure focus, (Connelly et al., 1994; Gadian et al., 1994; Matthews et al., 1990) including in non-lesional cases (Connelly et al., 1998) and the glutamine plus glutamate (Glx) to NAA and Choline ratios can correctly identify the epileptogenic zone in patients with temporal lobe epilepsy (Savic et al., 2000). In an MRS study of focal cortical dysplasia (Simister et al., 2007), patients had elevated levels of glutamine/glutamate and reduced levels of NAA compared with controls. Other studies have shown that higher concordance between MRS abnormalities and region of surgical resection is associated with better surgical outcomes (Pan et al., 2013).

For measurement of glutamate, PRESS and MEGA-PRESS techniques have shown excellent in vitro agreement between actual and measured glutamate concentrations, as well as good repeatability at 3T (van Veenendaal et al., 2018). Clinically, elevated glutamate concentrations have been associated with seizures in a population of glioma patients with epilepsy (Leir et al., 2015) and another study showed a correlation between glutamine/glutamate (Glx) levels on MRS and frequency and duration of seizures in neonatal hypoxic-ischaemic encephalopathy (Pu et al., 2008).

Glutamate is difficult to separate from glutamine at 1.5T; at higher field strengths, improved separation is possible although factors such as increased B0 and B1 inhomogeneities may pose technical challenges (Vargas et al., 2018). Other challenges cited include the inadequacy of single voxel spectroscopy due to the potential dilution of small abnormalities by partial volume effects and the need to cover wide brain territories (Pan et al., 2013). Therefore, MRS is yet to have an established role in the routine management of what is such a heterogeneous group of disorders (Pan and Kuzniecky, 2015).

Glutamate can be measured non-invasively with an ultra-high field (7T) MRI technique called Glutamate Chemical Exchange Saturation Transfer (*GluCEST*), developed at the University of Pennsylvania (Cai et al., 2012). CEST techniques exploit the ability to image metabolites based on their water protein exchange rates (Ward et al., 2000). The main advantage of GluCEST over MRS is its ability to provide increased spatial and temporal resolution (Cai et al., 2012). In vitro, the GluCEST effect is proportional to glutamate concentration at pH 7 and there is consistency between GluCEST contrast and both ^{1}MRS glutamate signal and PET imaging of the distribution of glutamate receptors. This technique has been used to localise epileptic foci in non-lesional focal epilepsy (Davis et al., 2015) but has not been used to study a human stroke population. Neal et al. recently demonstrated that increased peritumoural GluCEST contrast is associated with both recent seizures and drug refractory epilepsy in patients with glioma (Fig. 2) (75).

2.6. Potential problems with the glutamate hypothesis

Despite the strong evidence implicating glutamate-related excitotoxicity in the pathogenesis of stroke, experimental treatment with glutamate antagonists has proved ineffective to improve functional outcome, although the impact on development of seizures was not studied. There are several explanations put forward for this failure, discussed in depth by Di Renzo et al. (2009). Several of these theories may be relevant to our understanding of post-stroke epileptogenesis.

One problem is the extent of release of glutamate in the ischaemic core compared with the ischaemic penumbra and its association with epileptogenesis: experimental data suggests it is the ischaemic

penumbra rather than the core that is responsible for epileptogenesis (DeLorenzo et al., 2007), consistent with the hypothesis that dead neurons do not generate epileptiform discharges, although in animal models of ischaemia the extent of elevation of extracellular glutamate levels is significantly higher in the core (Obrenovitch et al., 1993). Furthermore the toxic effect of glutamate may be offset by the neuroprotective effect of extracellular acidity in the setting of acute stroke (Kaku et al., 1993); it is possible though that tissue acidosis may protect against infarct growth but not against the cellular excitability and altered calcium homeostasis observed in the structurally intact penumbra. It is also unclear if the deleterious effects of glutamate toxicity are in any way offset by the concurrent rise in extracellular levels of the inhibitory neurotransmitter GABA (Matsumoto et al., 1993).

2.7. Summary and future directions

Stroke is one of the most important causes of acquired epilepsy, particularly in the adult population. Identifying cellular and molecular factors relevant to the development of post-stroke epilepsy therefore represents an area of great clinical need. An elevation in extracellular cerebral glutamate levels has been demonstrated post-stroke, particularly in the hyper-acute phase, in both animal and human studies. While early studies showed higher glutamate levels were associated with worsening of infarct growth, more recent Magnetic Resonance Spectroscopy data suggests that glutamate may form part of a metabolic signature of hyper-perfused tissue which is associated with relatively better clinical outcomes after reperfusion therapy.

There is also significant evidence to support the role of glutamate in the initiation and propagation of seizure activity as well as the process of epileptogenesis. In this setting excessive glutamate activity may be mediated by a combination of aberrant signalling via glutamate receptors, down-regulation of glutamate transporters leading to decreased uptake of glutamate from the extracellular space, or enzymatic dysfunction. Furthermore, treatments that antagonise glutamate receptors or up-regulate glutamate transporters may attenuate this risk, although whether this applies to post-stroke epileptogenesis is unclear. There are thus far no studies that have correlated excessive glutamate levels in acute stroke with the development of epilepsy; only one small study has demonstrated an association between plasma glutamate levels in those with early post-stroke seizures compared with those without early seizures (Xie et al., 2016).

The advent of 7T MRI technology represents a new opportunity to study intracerebral glutamate levels following stroke in an in-vivo setting, both through Magnetic Resonance Spectroscopy and utilising the *GluCEST* technique. Accordingly we will be able to better understand the temporal changes in glutamate levels after a stroke and identify the optimal time point for glutamate receptor antagonism as an anti-epileptogenic strategy. One caveat is that *GluCEST* contrast is pH dependent and needs to be interpreted in the setting of tissue acidosis, and non-invasive imaging techniques cannot necessarily differentiate between extracellular and intracellular glutamate content. The non-selective, competitive AMPA receptor antagonist perampanel represents a possible anti-epileptogenic agent; it has shown neuroprotective effects in animal models and has an established role in the treatment of epilepsy.

While the search for anti-epileptogenic therapies has thus far been a challenging one, developing a better understanding of the pathogenesis of post-stroke epileptogenesis and implementing potential neuro-protective strategies represents an opportunity to improve the quality of lives of those suffering from a common and serious long term sequela of stroke.

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Declaration of Competing Interest

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