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REVIEW

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Clinical pharmacological issues in the development of acute stroke therapies

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The demonstration of the ischaemic penumbra in animal models and the effectiveness of reperfusion therapy in humans led to considerable optimism for neuroprotection in acute stroke. Initial experience with failure of phase II and III trials led to the STAIR recommendations for pre-clinical and clinical studies. Review of pre-clinical studies suggests that selection of agents for clinical development may not have been optimal. The neuroprotective agent NXY-059 fulfilled pre-clinical and many clinical STAIR criteria but a second large phase III study failed to demonstrate any benefit. Many of the STAIR criteria have not been fulfilled in the development of recent neuroprotective agents. Other issues not addressed include the use of animal models more reflective of older stroke patients with physiological derangement, demonstration of drug distribution to the proposed site of action in humans, selection of patients with salvageable tissue, achieving very early treatment, refinement of measurement of neurological impairment and disability, and physiological optimization in proof of concept human studies. Increasing the number and quality of clinical centres undertaking acute stroke research, use of surrogate imaging markers and adaptive dose designs in phase II trials could improve the likelihood of identifying an effective neuroprotective. Neuroprotection in acute stroke remains a significant challenge but has not been clearly shown to be ineffective. Given the profound burden of stroke and limited applicability of reperfusion to currently at best 10% patients, further proof of concept studies of neuroprotection remain indicated with careful review of pre-clinical data and more rigorous phase II trial design.
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Abbreviation: STAIR, Stroke Therapy Academic Industry Roundtable

Introduction

Twenty-five years ago, the medical community generally considered that acute stroke was an untreatable acute medical problem. A standard medical textbook stated, 'There is probably little that medical treatment can do to alter the immediate prognosis of stroke. Both fibrinolytic drugs and anti-coagulation increase the risk of intracranial bleeding and should usually not be used.' (Warlow, 1983). Since then, views about the potential for acute neuroprotective stroke treatments to improve prognosis have swung from nihilism through optimism to profound scepticism. Astrup *et al.* (1981) described the existence of the ischaemic penumbra prompting the undertaking of a large number of studies of neuroprotection, which demonstrated the effectiveness of reducing infarct size in rodent models using permanent or transient middle cerebral artery occlusion (O'Collins *et al.*, 2006). Studies of reperfusion therapy with thrombolytic agents also demonstrated time-dependent effectiveness of thrombolytic therapy in reducing infarct size (Zivin *et al.*, 1988). However, in the last 10 years, a succession of failed clinical studies has led to considerable scepticism as to whether neuroprotection is an achievable goal in man. In this context, it is helpful to reflect on the positive advances in acute stroke treatment, which demonstrate reversal of the view in the early 1980s that the outcome from acute stroke is not modifiable. A key observation was a meta-analysis of trials of stroke units, including acute and rehabilitation units, which demonstrated that organized stroke care compared with usual medical care reduced death and disability (Langhorne *et al.*, 1993). Although not directly relevant to the development of drug therapies, this demonstrated that the outcome from stroke could be improved by medical practice. The NINDS (National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group) study of alteplase in acute ischaemic stroke reported in 1995 confirmed the efficacy of thrombolysis in carefully selected patients with acute ischaemic stroke within a 3 h window, with time dependency very similar to that seen in animal models (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). Large trials of aspirin commenced within 48 h of stroke onset demonstrated a

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small but important reduction in recurrent stroke and improved long-term outcomes in a combined analysis of the two large trials (Chen *et al*., 2000). Some lessons from these successes have not been fully incorporated into the development of neuroprotection in acute ischaemic stroke. More recently, a phase II trial of recombinant factor VIIa in primary intracerebral haemorrhage successfully achieved demonstration of proof of the concept that early treatment commenced within 3 h of symptom onset could reduce haematoma expansion (Mayer *et al*., 2005). However, no benefit in disability was observed in a subsequent phase III clinical trial study (presentation 2007 European Stroke Conference), possibly due to the study being underpowered or to adverse thrombotic events negating any benefit from reducing haematoma expansion. Thus, even when proof of concept has been demonstrated, this may prove difficult to translate into clear clinical benefits demonstrable in phase III trials.

Reperfusion therapy

Although alteplase is now licensed for the treatment of stroke in North America and Europe and was recently approved by the National Institute for Health and Clinical Excellence (2007) as a recommended cost-effective therapy, this achievement was beset by many early difficulties. Indeed, controversies still exist around the risk/benefit ratio in certain groups of patients such as the very elderly. Initial trials of the thrombolytic agent streptokinase were stopped prematurely because of an increase in early mortality associated with an increase in intracerebral haemorrhage. Small-dose finding studies suggested that alteplase had an acceptable safety profile and led to the positive two-part NINDS late-phase clinical trial of alteplase in acute ischaemic stroke (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). This trial used stratified randomization in 0–90 and 90–180 min cohorts to achieve an average time to treatment of 90 min from symptom onset. Three further similar sized clinical trials with 5–6 h time windows failed to achieve significant results on the primary outcome (Hacke *et al*., 1995, 1998; Clark *et al*., 1999). Although significant criticisms were made of the NINDS trial with imbalance in the severity of stroke patients between active and control groups in the early and late time periods, an independent analysis and individual patient meta-analysis indicated that the primary reason for the positive result achieved in NINDS was the very early treatment compared with other trials (Hacke *et al*., 2004; Ingall *et al*., 2004). The large phase IV Safe Implementation of Thrombolysis in Stroke-Monitoring study has confirmed that the outcomes and haemorrhagic complications with thrombolytic therapy in the 3 h time window are as good as those observed in the randomized controlled trials (Wahlgren *et al*., 2007). The benefits of thrombolytic therapy with intravenous alteplase are critically time dependent as seen in animal models of reperfusion. This led to an appreciation of the importance of achieving rapid early treatment in clinical stroke trials. Remarkably, 12 years since the NINDS trial was reported, no other clinical stroke trials have achieved such a short average time to treatment.

Clinical issues in acute stroke

There are a number of important clinical issues that are important in the design and conduct of clinical stroke trials when seeking to translate the results seen in animal studies. Unlike the standard animal model of permanent or temporary middle cerebral artery occlusion, clinical stroke is very heterogeneous. In 10% of cases, stroke is due to intracerebral haemorrhage rather than cerebral ischaemia with very different pathophysiology without an ischaemic penumbra in cerebral haemorrhage. In patients with ischaemic stroke, occlusion may be in large or small vessels and secondary to *in situ* thrombosis, artery-to-artery embolism or cardiac embolism, and affect very different areas of the brain. The consequences of small-vessel occlusion very likely differ from large-vessel occlusion with respect to the effect of neuroprotection, and good animal models of small-vessel occlusion have not been developed. Stroke occurs predominantly in older patients with an average age over 70 years in developed countries. In contrast, experimental models nearly always use young animals. Little is known of the effect of age on neuronal vulnerability and collateral circulation, which could modify the response to a neuroprotective. In addition, stroke patients often have significant comorbidities such as cardiac or pre-existing neurodegenerative disease, which may confound clinical outcome assessment and the response to a neuroprotective agent. A further important difference between patients and controlled animal studies is the presence of considerable physiological variability with frequent elevations and variability in blood pressure, glucose, temperature and oxygenation in contrast to experimental models where animals are anaesthetized and physiological parameters controlled.

Experimental stroke studies are almost entirely undertaken in young animals, whereas stroke predominantly occurs in older people with vascular disease and other comorbidities, particularly diabetes and hypertension. The average age of acute stroke patients in developed countries is mid-70s, and ageing demographics and improved prevention strategies in middle age are likely to result in this figure increasing to 80 years. Some studies of neuroprotection agents have been performed in older animals (Davis *et al*., 1995; Schaller, 2007) and have suggested that efficacy may be reduced. Although the practicalities and cost of using older animals argue against using older animals routinely, more work examining age-associated differences in response to neuroprotection in animal models would be of value in informing the design of early phase clinical studies and the need for experimental data in older animals. The response to neuroprotection might be reduced in older subjects because of increased neuronal susceptibility to ischaemia and impaired collateral circulation to the ischaemic penumbra in older patients with concomitant vascular disease, although the limited evidence available, suggesting efficacy of reperfusion therapy in the very elderly, would argue against these being major issues. However, more research is needed in both animals and

humans to increase understanding of the importance of age as an influence on response to neuroprotection.

A further confounding issue is that as recovery from moderate or severe stroke occurs over weeks and months, outcomes from acute stroke treatments are usually assessed at 90 days following the acute event. Substantial evidence (Langhorne *et al*., 1993) demonstrates that stroke units, rehabilitation or combined acute/rehabilitation, have an important influence on outcome reducing death and disability. Audits of clinical services indicate substantial variation in the quality of care and outcomes across different centres (Intercollegiate Stroke Working Party, 2007). Analysis of outcomes from different countries within clinical trials (Weir *et al*., 2001; Gray *et al*., 2006) demonstrates considerable variation in outcomes between different countries. Even within individual centres, the quality of care that patients receive may be highly variable if all patients do not access organized acute and rehabilitation stroke care. In multicentre studies, these factors produce considerable confounding and reduce study power. The influence of variation in stroke care during the period before final outcome assessment could be reduced with standardization of access to organized stroke unit care and the introduction of standardized protocols in the management of physiological variables. However, such protocols are difficult to agree and implement across different health-care systems.

Systematically reviewing preclinical data and STAIR criteria

Experimental preclinical studies in animal models have a number of roles, including identification of therapeutic targets such as the ischaemic penumbra, general toxicity testing a requirement prior to administration of a new chemical entity to humans and generation of efficacy dose–response curves to inform the selection of an appropriate range of doses for phase II studies in humans. In addition, animal models can be used to study the influence of ageing and physiological variability on drug response, as discussed earlier.

Following the failure of many clinical neuroprotective trials in 1999, a group comprising academic and industry representatives, the Stroke Therapy Academic Industry Roundtable (STAIR), developed a series of recommendations for standards in preclinical neuroprotective and restorative drug treatment. These can be summarized in 10 key points shown in Table 1. Many of the STAIR criteria are intuitively correct but because of the absence of successful neuroprotection trials in man, there is inevitably little evidence supporting them as predictors of clinical efficacy. The criteria will only be clearly validated when a neuroprotective agent shown to have efficacy in humans has been shown to meet them. As discussed below, the preclinical criteria have also undergone some expansion and development since their initial publication.

The systematic review of clinical studies is now seen as an important step to inform decisions about appropriate future research but has not been an approach used in reviewing experimental studies and the choice of agent to take forward into early phase human studies. O'Collins *et al*. (2006) have undertaken an extensive review of experimental stroke treatments performed between 1957 and 2003. They identified 912 drugs studied in neuroprotection experiments and 14 drugs taken through to clinical trials (O'Collins *et al*., 2006). They developed a 10-point scoring system based on the STAIR criteria above, adding in focal model testing in old or diseased animals to assess the quality of preclinical data for individual agents. Inclusion of these additional criteria is supported by a systematic review of the animal evidence base for FK506, involving meta-analysis from 1759 animals, which found that efficacy was higher in models using ketamine anaesthesia and temporary ischaemia but lower in animals with comorbidities.

Table 1 Preclinical STAIR criteria

- Blinded-randomized testing in rodents with models of focal cerebral infarction
- Replication of results in at least two laboratories with adequate physiological monitoring
- Studies in permanent and reperfusion models
- Testing in male and female animals
- Rat behavioural and histological studies for at least 1 month after focal occlusion
- Use of a route of drug administration that is feasible for clinical development, to ensure agents cross the blood–brain barrier
- Toxicological studies in several species, including intact animals and animals with stroke
- Testing in primate models, including behaviour, sensorimotor and cognitive function, for drugs showing promise in rat models
- Careful dose–response studies
- Time window studies showing benefit at delayed time points after stroke onset

Surprisingly, they reported that drugs taken through into clinical trials were not more effective in experimental models than in those that were not. They concluded that greater rigour was required in the conduct, reporting and analysis of the results of experimental model data to improve the selection of drugs to take through to early phase studies in humans. In the systematic review of FK 506, the reported study quality was modest, and efficacy was lower in high-quality studies. The authors concluded that there is substantial efficacy for FK506 in experimental stroke but that the reported estimate of effect size might be too high because of study quality and possible publication bias (Macleod *et al*., 2005).

Although the validity of the STAIR preclinical criteria have been questioned, these criteria need to be considered alongside the quality of the subsequent clinical early phase studies for which separate STAIR criteria were developed. O'Collins *et al*. (2006) identified four treatments that satisfied all their 10 STAIR preclinical criteria: hypothermia; NXY-059, a purported free radical scavenger; ARL15896, an N-methyl-D-aspartic acid (NMDA) antagonist (also referred to as AR-Ra5896AR) and basic fibroblast growth factor. For only the non-drug intervention, hypothermia has evidence of neuroprotection in humans being demonstrated. Mild-to-moderate hypothermia with cooling to 33°C initiated within 2 h of cardiac arrest (Bernard *et al*., 2002) increased the likelihood of a good outcome (26–49%). The clinical development of AR-R15896AR was discontinued after phase II studies demonstrated adverse effects of dizziness,

vomiting, nausea, stupor, agitation and hallucinations at plasma concentrations just after achieving the neuroprotective plasma concentrations (Lees *et al.*, 2001; Diener *et al.*, 2002). These adverse effects were seen with other NMDA antagonists taken through to clinical development and were a limiting factor in optimizing dosing (Muir and Lees, 1995). The development of NXY-059 is discussed in detail because it fulfilled STAIR preclinical criteria and was studied in two large phase III trials.

Two large phase III trials of NXY-059 have been performed in approximately 5000 acute ischaemic stroke patients within 6 h of stroke onset. Although the initial Stroke-Acute Ischaemic NXY Treatment (SAINT) 1 study (Lees *et al.*, 2006b) suggested a clinical benefit on a disability end point, this was not confirmed in the larger subsequent SAINT II trial (Shuaib *et al.*, 2007). Following the negative SAINT II trial, Savitz (2007) concluded that the development of NXY-059 followed many of the STAIR guidelines (Savitz and Fisher, 2007), but in another publication he argued that a more critical, cautious review suggests that the preclinical data were not robust without published data replicating neuroprotective effects after 3 h in the transient occlusion model and without data in a clinically relevant model of embolic stroke. Savitz suggests that the STAIR preclinical criteria require revision to address choice of animal model, control studies, measurement of cerebral blood flow to document occlusion, reproducibility of treatment effects at late time points, choice of behavioural tests, use of gyrencephalic primate models and that clinical investigators should design studies that better match preclinical data, a point discussed later. Inevitably, a review of the STAIR preclinical criteria is needed, but such a review probably needs to take a broader translational perspective and consider how the design of early phase clinical studies can more closely mirror preclinical studies and vice versa.

Surrogate markers of therapeutic effect

The failure to translate the efficacy of neuroprotective drugs into clinical benefits in phase III trials even for agents meeting all the STAIR preclinical criteria reflects the continuing challenge to identify reliable surrogate markers to demonstrate proof of concept in early phase studies of neuroprotectants. Surrogate markers have been developed to evaluate reperfusion therapies for acute stroke using angiographic or Doppler demonstration of recanalization (Alexandrov *et al.*, 2004; Hacke *et al.*, 2005), which has reduced but not removed the risk of failure in later phase studies. The Desmoteplase in Acute Ischemic Stroke Trial phase II studies of desmoteplase used magnetic resonance (MR) perfusion/diffusion and MR angiography to recruit patients in the 3–9 h time window with diffusion–perfusion mismatch, suggesting that salvageable ischaemic penumbra was present. Both studies demonstrated a dose–response in the proportion of patients achieving recanalization assessed by MR angiography and an associated improved functional outcome in patients who reperfused (Hacke *et al.*, 2005; Furlan *et al.*, 2006). The association between early reperfusion and a good clinical outcome has been shown in other studies of intravenous and intra-arterial thrombolysis. A third later phase II study then allowed the use of either MR diffusion–perfusion or CT perfusion to identify patients 3–9 h following acute stroke onset with 'mismatch' but found no better outcome with desmoteplase (data presented at 2007 European Stroke Conference). It is unclear whether the disparity between these studies is due to the play of chance or problems with CT perfusion in reliably identifying patients with salvageable tissue. The second study did not include assessment of the effects on recanalization through follow-up MR angiography or CTA and makes interpretation of the study very difficult and illustrates the importance of keeping markers of drug effect throughout phase II studies and preferably in at least some of the phase III studies. Lessons can be learnt from the experience of developing reperfusion therapy for myocardial infarction where the effect of thrombolytic agents in development on recanalization rates were well defined before and during phase III trials (Binaghi *et al.*, 1987).

The use of advanced MR or CT imaging to identify patients for neuroprotective trials has been discussed but not yet applied to the development of neuroprotective drugs. A collaborative analysis of MR diffusion–perfusion mismatch and diffusion lesion growth from acute stroke patients across a number of centres has suggested that 99 patients would be needed in each arm of an MR-based study to detect a 20% absolute reduction in the infarct expansion ratio (Phan *et al.*, 2006) at conventional levels of statistical significance. Although this approach has many attractions, there are a number of problems that need to be addressed. One barrier to the adoption of this approach is the limited number of centres with access to hyper acute advanced MR or CT imaging, although access is improving and The Desmoteplase in Acute Ischemic Stroke Trial and other ongoing thrombolytic trials indicate that this approach is feasible. Another barrier to this approach is the desire of pharmaceutical companies and clinicians treating acute stroke patients to use a neuroprotective agent that is effective in all subtypes of ischaemic stroke. This leads to pressures to develop simple inclusion criteria for phase III studies. Accurate differentiation of lacunar and cortical ischaemic strokes is often not possible in the initial clinical assessment of patients who may have normal unenhanced CT brain imaging. Using advanced imaging studies in early phase studies argues for such criteria to be then included in initial phase III studies.

Clinical trial development and STAIR criteria

Following the development of the STAIR criteria for preclinical neuroprotectant studies led to the subsequent development at a second STAIR group meeting in 2000 of recommendations for phase II and III clinical trials of neuroprotective drugs in stroke. These recommendations were made at a time when a number of drugs had been taken through development with either significant side effects with limited pharmacokinetic data or with long time windows up to 12–24 h, following symptom onset in some phase II studies. The STAIR II criteria for designing phase IIb stroke trials are shown in Table 2. There has been much less

Table 2 Phase IIb programme STAIR II criteria

- Route of administration
- Dose range
- Time from stroke onset to initiation of treatment
- Pharmacokinetic profile
- Side effects and their frequency
- Drug interactions
- Drug distribution to the proposed site of action
- Refinement and identification of the target population
- Established therapeutic activity through evaluation of clinical and/or surrogate markers

focus on and discussion of the clinical STAIR criteria in comparison to the preclinical criteria. Some of these criteria are still not being fulfilled in phase II development programmes, in particular the demonstration of drug distribution to the proposed site of action, refinement and identification of the target population, and the establishment of therapeutic activity through the evaluation of clinical and/or surrogate markers. The STAIR group emphasize that phase IIb study end points should be easy to measure, reproducible, valid, clinically meaningful and resistant to bias. This remains a problematic area for phase II studies in neuroprotection because of the lack of positive neuroprotective trials to select validated surrogate end points. Some imaging methods such as MR diffusion/perfusion are not easy to measure. Many clinical measures, such as changes in neurological impairment such as National Institutes of Health Stroke Scale (NIHSS) change, show poor interindividual agreement, although agreement for full functional recovery (NIHSS scores 0 or 1) is better and has been used as an outcome in some phase II studies. A decision has to be made how to handle impairment scores in patients who die before final study follow-up. Usually, these patients are categorized as having the maximal NIHSS score (42), although the last observation carried forward an approach that can also be used. The inclusion of high scores for dead patients results in a bimodal skewed distribution of NIHSS change scores and requires nonparametric analysis (Lees *et al*., 2006a). If the development of NXY-059 that was assessed in the largest number of acute ischaemic stroke patients is considered, four of the nine STAIR IIb criteria can be considered to have not been fulfilled. A 24 h time window was used in the phase II dose-ranging studies despite the intended time to treatment for the phase III studies being 0–6 h. Despite this, the assessment of disability in a phase II study that showed a positive trend in the active treatment group was interpreted as possible efficacy (Lees *et al*., 2003). Drug distribution to the proposed site of action had not been demonstrated in humans, although the site of action in animal models had not been established. Refinement and identification of the target population was not achieved in the phase IIb studies with patients with cerebral haemorrhage and very mild ischaemic deficits being included. Therapeutic activity for NXY-059 was not achieved through either clinical or surrogate markers. The importance of these criteria being fulfilled was recognized by researchers, but the methods to demonstrate drug distribution to the site of action and validity of surrogate markers were not available. From a clinical pharmacological perspective, a key issue in drug development is to understand from animal models the site of action and to demonstrate drug distribution to this site. In the case of most neuroprotectants, the proposed site of action was neurones in the ischaemic penumbra. NXY-059 was known to have poor penetration across the blood–brain barrier in preclinical models and demonstrated no adverse central nervous system effects in humans (Dehouck *et al*., 2002). However, blood–brain penetration would not be necessary if the site of action was at the endothelial cell, as first suggested by Kuroda *et al*. (1999). The development of NMDA antagonists had usually demonstrated adverse effects of agitation and hallucinations, indicating evidence of drug activity in the brain, and these or the failure to achieve putative plasma neuroprotective concentrations have been considered the main cause of failure in phase III studies. Similarly, the blood–brain barrier penetration and CSF concentration profiles following intravenous plasma loading were not known for the glycine antagonist gavestinel, which failed to show efficacy in clinical phase III studies and in an MR imaging substudy (Sacco *et al*., 2001; Warach *et al*., 2006). One approach being used is to study plasma and CSF pharmacokinetics in patients with subarachnoid haemorrhage who are at risk of delayed cerebral ischaemia and who have external ventricular drains placed that enable CSF drug concentrations to be analysed. Understanding the rate at which a neuroprotective drug transfers into the CSF drug compartment is key in designing appropriate time windows and dosing regimens for phase II studies.

A well-recognized confounding factor in assessing the response to a neuroprotective drug is whether reperfusion occurs within the first few hours. This phenomenon is well demonstrated in preclinical models where the extent of infarction is greater in permanent occlusion models and the volume of tissue salvaged by neuroprotective agents is reduced. Some clinical trials have used thrombolytic treatment as a surrogate marker for reperfusion, although it is not an accurate measure, as around one-third of patients treated with intravenous alteplase do not reperfuse and some patients who are not treated with thrombolysis reperfuse spontaneously. In early phase studies determining the site of vessel occlusion and establishing whether reperfusion occurs within the first few hours, through MR angiography or Doppler studies might enable proof of concept of neuroprotective effects to be demonstrated in the group of patients who reperfuse.

The 2000 STAIR group also made nine recommendations for the design of phase III acute stroke trials (Table 3). In the phase III studies of NXY-059, patients had to have limb weakness, which included both cortical and subcortical lacunar strokes. This inclusion was justified on the basis that protection of both white and grey matter was seen in animal models unlike previous neuroprotectants where preclinical studies had shown little neuroprotection in white matter. Assessing the development of NXY-059, which was assessed in the two largest phase III trials to date, it is questionable whether four of the nine recommendations were fulfilled. The time window for drug initiation was 6 h with an average time to treatment of 4 h following symptom onset, arguably too long when the neuroprotective effects at 6 h after onset were observed to be absent in some animal

Table 3 Phase III programme STAIR II criteria

- Dose selection based on phase 1 and II data plasma concentrations
- Time window for drug initiation
- Patient selection based on mechanisms of action
- Outcome measures
- Severity of stroke population to be studied
- Length of follow-up period
- Use of surrogate markers to provide support for drug efficacy
- Prespecification of covariate analysis
- Fostering of appropriate and effective relationships between sponsors, academics and investigators

models (Kuroda *et al*., 1999). However, the average time to treatment was under 4 h in the larger SAINT II trial, which was consistent with the preclinical findings, which had shown substantial neuroprotection out to 4 h in a number of models and species. Subgroup analysis of the SAINT II trial demonstrated no benefit in the patients who received treatment within 4 h. Although the free radical trapping properties of NXY-059 were well documented, there was considerable uncertainty as to the site of action and mechanism by which NXY-059 produced neuroprotection in animal models. Following primate studies, Marshall *et al*. (2003) stated that their studies raised questions regarding the mechanism of action of NXY-059, commenting that it was unclear whether it was effective at trapping radicals in the brain. They stated that NXY-059 maintained Akt activation and inhibited cytochrome *c* release after ischaemia and that it may interact directly with penumbral neurons or interact with the endothelial cell to alter signal transduction pathways. Subsequent work has indicated that NXY-059 has no free radical trapping properties in a cell culture model of neurotoxicity (Hainsworth *et al*., 2008) and most probably acts at the neurovascular unit (Fisher *et al*., 2006) where brain penetration would not be a requirement for access to the site of drug action. Overall, these findings indicate that the site and mechanism of action of NXY-059 was not clearly understood in animal models at the time the agent was developed in clinical studies. Considerable discussion followed publication of the SAINT 1 trial with the use of a co-primary outcome of modified Rankin scale and change in the NIHSS (Lees *et al*., 2006b) with a positive outcome on the modified Rankin scale but not NIHSS change. Publication of detailed analysis plans prior to publication would avoid some of the controversies that arise. There were no surrogate imaging markers, which would have provided support for drug efficacy.

Lessons from previous clinical trials

Despite the failure of multiple neuroprotective drugs in clinical trials, a number of key lessons have been learnt. The rush from bench to bedside is recognized to need change. Future neuroprotective agents need to be evaluated more rigorously in multiple animal models and robust protection needs to be demonstrated in 3–6 h time windows in animal models. More thought needs to be given to taking knowledge at the bedside back to the bench, a process sometimes referred to as 'reverse translation'. The importance of including a short-time window in both phase II and III clinical studies is now recognized as this increases the likelihood of demonstrating a positive effect on surrogate outcome and a significant effect on improving clinical stroke outcomes. Three-hour time windows with pre-inclusion imaging are now feasible. The importance of rapidly achieving plasma concentrations that are associated with neuroprotection in animals is now well recognized and incorporated into recent development programmes (Jonsson *et al*., 2005). The difficulties in managing serious CNS adverse effects are now well recognized and agents with obvious serious dose-dependent toxicity are no longer being taken through to phase III. Progress is being made with the standardization of both imaging and clinical outcomes. The importance of having a structured assessment and training in clinical outcome assessments is increasingly recognized (Wilson *et al*., 2005). Finally, the importance of undertaking adequately powered studies is now acknowledged. Initially, the over-optimistic belief that neuroprotective agents would produce clinical benefits as effective as reperfusion therapy has been tempered, and phase III studies are now being designed to detect 3–5% increase in good functional outcome using the modified Rankin scale.

Challenges for future clinical stroke trials

Despite this progress from a clinical pharmacological perspective, the design of early phase studies could be further improved. Larger dose-ranging studies with putative neuroprotectant drugs need to be conducted in a population of patients more representative of the patients who would be treated in phase III trials (early onset, significant deficit). More consideration should be given to incorporating adaptive dose designs at this phase. This approach was successfully implemented with a Neutrophil Inhibitor Factor phase IIb study, with the ongoing analysis resulting in early termination of the study with a demonstration that the drug failed to improve neurological recovery across a range of doses (Krams *et al*., 2003). Further investment in the development of surrogate imaging markers appears warranted and the MR diffusion/perfusion mismatch approach will be assessed in its validity as a marker of benefit for reperfusion therapies in ongoing studies.

Much thought should be given in phase II studies to standardizing and quality assuring the 'clinical laboratory' as is undertaken in preclinical studies. Protocol-driven management of blood pressure, temperature, plasma glucose levels and oxygenation might reduce some of the variability between centres. Further research as to the effects of changing these parameters in the hyperacute setting is required. Elevations in blood pressure and glucose are known to be associated with a worse outcome and increased risk of bleeding with thrombolytic therapy, but whether lowering these acutely improves outcome or reduces the risk of bleeding is unknown. The Glucose Insulin in Stroke Trial reported that no benefit of glucose lowering commenced within 24 h of stroke onset for 24 h but the glucose lowering effect was modest. It also reported that a heterogeneous group of patients were included and that the duration of treatment was only for 24 h (Gray *et al*., 2007). As preclinical

models suggest that these parameters may exert substantial influence on outcome and drug effects, a standard approach to managing these in the clinical setting appears warranted. Similarly, standardization or the setting of a minimal level of quality of acute and rehabilitation care across centres in multicentre studies that have 30- or 90-day clinical outcomes should reduce the sample size needed to detect a treatment effect. Finally, given the importance of time to treatment in maximizing treatment effects, early phase clinical studies need to be designed with short-time windows and should attempt to replicate the conditions under which neuroprotective effects are seen in preclinical studies. Preclinical models need to be more reflective of the typical older stroke patients with physiological derangement, and the quality, reporting and analysis of preclinical studies should be improved. *In vitro* human studies using cell culture or tissue slices could be used more often to confirm the likely mechanism of action seen in preclinical models. Demonstration of drug access through the blood–brain barrier to the putative site of action is a critical step in early phase human studies for agents that is believed to act directly on penumbral neurons, and it may require studies in other patient groups undergoing neurosurgery or intracranial pressure monitoring where CSF can be sampled. Where the site of action is the penumbra, the selection of patients with salvageable tissue is key and imaging-based selection currently appears to offer some promise. Physiological optimization of blood pressure, glucose and oxygenation should be considered through managed protocols in proof of concept human studies. The incorporation of adaptive dose designs should be considered in phase II studies. In phase III, very early treatment through involvement of pre-hospital services may be appropriate, although the selection of patients then has to be entirely on clinical criteria without brain imaging. This approach is being used in the ongoing Field Administration of Stroke Therapy-Magnesium study examining the effect of magnesium administered in the pre-hospital setting by paramedics within 2 h of symptom onset (Saver *et al.*, 2004). Further standardization and refinement of clinical measures of neurological impairment and disability are needed for use in multicentre stroke studies. The number of clinical centres undertaking acute stroke research needs to increase and the quality of data needs to be collected carefully monitored if large-scale multicentre studies are to robustly demonstrate important but possibly modest effects of neuroprotection.

In summary, the development of neuroprotection has proved more challenging than anticipated. However, some core principles of clinical pharmacology have not been applied to most drugs developed because of difficulties in imaging and complexities of standardizing the clinical management of patients. The failure to translate preclinical findings into humans mandates closer dialogue and working between preclinical scientists and acute stroke triallists if neuroprotection is to deliver its promise.

Conflict of interest

The author's institution has received research grants from a number of companies for clinical trials in acute stroke, including AstraZeneca (manufacturer of NXY-059) and Boehringer Ingelheim (manufacturer of alteplase); the author has received consultancy and speaker honoraria relating to acute stroke treatment from AstraZeneca, Bristol Myers Squib/Sanofi and Boehringer Ingelheim.

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