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Therapeutic strategies for the treatment of stroke

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Acute ischaemic stroke is a major health problem with no effective treatments apart from the thrombolytic recombinant tissue plasminogen activator (rt-PA), which must be given within 3 h of stroke onset. However, rt-PA increases the risk of symptomatic intracranial haemorrhage and is administered to <5% of stroke patients. New perfusion-enhancing compounds are in development but the risk:benefit ratio remains to be determined. Many neuroprotective drugs have been studied but all those that reached clinical development have failed to demonstrate efficacy. However, adherence to recently published guidelines on preclinical development has resulted in one novel compound (NXY-059) demonstrating efficacy in a Phase III trial, providing encouragement for the validity of the concept of neuroprotection. There are a variety of new neuroprotective compounds in the early stages of investigation and some could prove clinically effective, provided appropriate preclinical development guidelines are observed.

Over the years a story has circulated that a distinguished neurosurgeon commented in the early 1980s that if one desired to guarantee the failure of a drug in clinical trials then one should develop a compound for the treatment of stroke [1]. Whether this was actually ever said is unimportant, what is important is that it is a view that continues to be held by many clinicians and by an increasing number of people in the pharmaceutical industry. Indeed, one could argue that anyone holding this pessimistic view is vindicated by the available evidence. Despite ever-increasing knowledge of the biochemical mechanisms that occur in the brain following an ischaemic insult, and the availability of several diverse animal models of stroke, there are still no drugs that can be given to stroke patients soon after the onset of symptoms to minimize the subsequent neurological problems that will be experienced, other than the thrombolytic compound recombinant tissue plasminogen activator (rt-PA). Furthermore, even this drug can only be used to treat a very small proportion of patients and is itself not without risk. In addition, every one of ~50 neuroprotectant compounds that have reached clinical trial has failed because of lack of demonstrable efficacy or problems with toxicity (Table 1); there are currently no neuropro-

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TABLE 1

Some compounds that have failed in clinical evaluation for the treatment of acute ischaemic stroke[a]

Compound	Mechanism of action	Inclusion period (h)	Outcome (Phase)	Reason[b]
Selfotel	NMDA receptor antagonist	6	Negative (III)	Adverse events
Aptiganel	NMDA receptor antagonist	6	Negative (III)	Lack of efficacy
Gavestinel	NMDA glycine-site antagonist	6	Negative (III)	Lack of efficacy
Eliprodil	NMDA, polyamine site blocker	6	Negative (II)	Adverse events
Magnesium sulphate	NMDA, channel blocker	12	Negative (III)	Lack of efficacy
Cervene	Kappa opioid receptor antagonist	6	Negative (III)	Lack of efficacy
Lubeluzole	NOS inhibitor and Na^+ channel blocker	8	Negative (III)	Lack of efficacy
Fosphenytoin	Sodium channel blocker	6	Negative (III)	Lack of efficacy
BMS-204352	K^+-channel blocker	6	Negative (III)	Lack of efficacy
Calcium antagonists	Ca^{2+} channel antagonists	6–24	Negative (Meta-analysis)	Lack of efficacy
Enlimomab	Anti-ICAM antibody	6	Negative (III)	Lack of efficacy and adverse events
Citicoline	Cell membrane stabilizer	24	Negative (III)	Lack of efficacy
Clomethiazole	$GABA_A$ receptor mimetic	12	Negative (III)	Lack of efficacy
Tirilazad	Lipid peroxidation inhibitor	4–24	Negative (III) 6 trials	Lack of efficacy
Ebselen	Lipid peroxidation inhibitor	12–48	Negative (II)	Lack of efficacy
Repinotan	5-HT_{1A} receptor antagonist	6	Negative (IIb)	Lack of efficacy
ONO-2506	Astrocyte modulating factor	6	Negative (II)	Lack of efficacy in futility analysis
Trafermin	Basic fibroblast growth factor	6	Negative (II/III)	Lack of efficacy
UK-279,276	Neutrophil inhibitory factor	6	Negative (II)	Lack of efficacy in futility analysis

[a] Abbreviations: BMS-204352, [35]-[+]-[5-chloro-2-methexyphenyl]-1,3-dihydro-3-fluoro-6-[trifluoromethyl]-2H-indol-2-one; ICAM, intercellular adhesion molecule; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase.
[b] Lack of efficacy means that efficacy was not demonstrated.

tective drugs on the world market. Therefore, although we are not surprised by the continued pessimism, we will nevertheless argue in this article that such pessimism should now be tempered with a degree of hope because recent data have indicated that we might be on the verge of being able to successfully treat a significant portion of stroke patients with effective drugs.

It is indeed vital that we do not give up the fight to develop compounds to treat stroke despite the many years of setbacks. Stroke is a problem that affects >15 million people world-wide, is the leading cause of disability and third leading cause of death in major industrialized countries. It has been estimated that ~6 million people have died from stroke in 2005 and that >90% of these deaths will have occurred in less affluent countries. The overall death rate will increase in the next decade by 12% globally and 20% in low income families [2]. More than 30% of stroke survivors will have severe disability and it has been calculated that by 2015 over 50 million healthy life-years will be lost because of stroke [2]. In its recent report 'Preventing Chronic Diseases: A Vital Investment' (http://www.who.int/chp/chronic_disease_report/en/index.html) the World Health Organization tries to dispel the notion that stroke is a disease of affluence and is calling for global action to halt the pandemic of stroke. Clearly if even a small proportion of people who suffer a stroke are returned to relative normality, the improvement in the lives of individuals and their families will be substantial and the burden to society in social and economic terms will be substantially reduced [3].

Most strokes (~85%) are ischaemic; that is, they result from an occlusion of a major cerebral artery by a thrombus or embolism. This results in loss of blood flow and a major decrease in the supply of oxygen and nutrients to the affected region. The remaining strokes are haemorrhagic, where a blood vessel bursts either in the brain or on its surface. In China, however, the figures differ, with a higher proportion of patients suffering a cerebral haemorrhage [4].

Thrombolysis or neuroprotection?

Over the past 30 years substantial knowledge has been gained on the initial neurochemical changes that occur following an ischaemic insult (Figure 1), this sequence of events often being referred to as the 'ischaemic cascade'. At present there are two major approaches to the treatment of acute ischaemic stroke: thrombolysis, to try and restore blood flow to the compromised region, and neuroprotection, which involves the use of drugs to interfere with one or more of the mechanisms in the 'ischaemic cascade' and thus minimize the subsequent neurodegeneration. Only thrombolysis is in clinical use in most parts of the world.

The basis of thrombolysis is the dissolution of the clot (hence the popular name for thrombolytics of 'clot busters'), thereby inducing blood reflow and reperfusion of the affected ischaemic tissue. The value of reperfusion can be gauged in animal studies simply by examining the consequences of either permanent occlusion of the rat middle cerebral artery (MCA) or occlusion followed by reperfusion 2 h later. The infarct size is substantially less in the

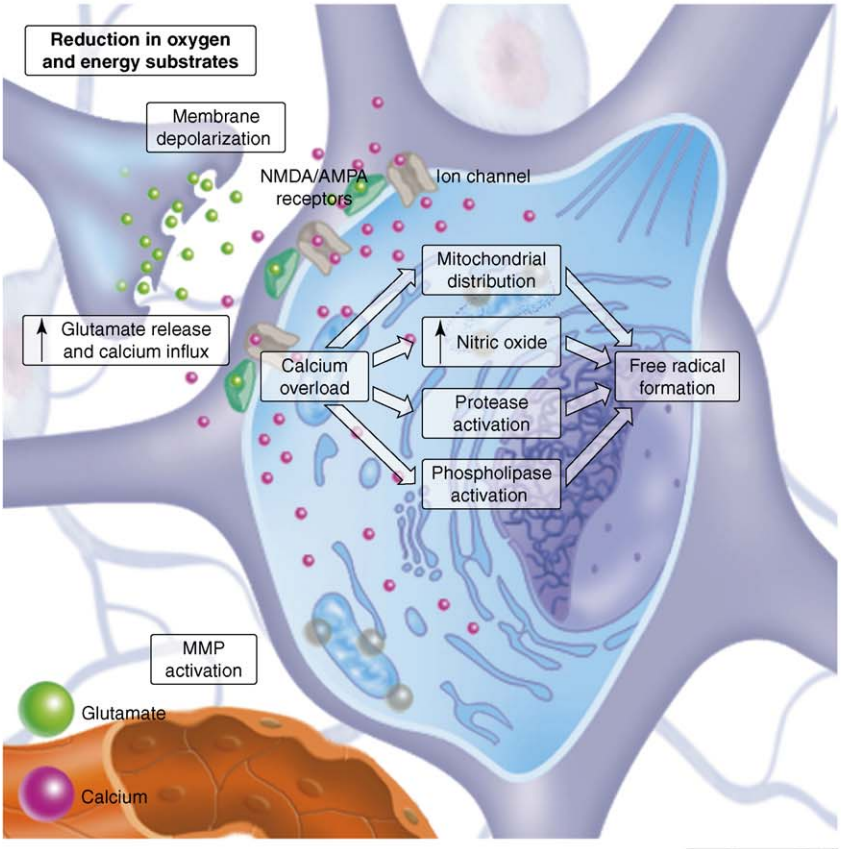

FIGURE 1

The initial 'ischaemic cascade'. The ischaemic cascade follows the onset of ischaemia and involves glutamate release followed by the other neurochemical changes shown. Biochemical changes in later stages of the ischaemic cascade are shown in Figure 2.

brains of rats subjected to the transient ischaemia compared to those subjected to permanent ischaemia [5]. However reperfusion also produces damage, primarily induced by free radical release [6–8], which can then initiate a series of biochemical changes that are further exacerbated by any endothelial damage that also occurs (Figure 2).

Although thrombolysis with rt-PA is clinically effective at treating acute ischaemic stroke [9], its use is limited by several factors. First, patients are required to undergo a computed tomography (CT) scan to exclude the possibility that they are suffering from a haemorrhagic stroke because thrombolytics are contraindicated in that situation. Because rt-PA must be given within 3 h, the delay caused by a CT scan, coupled with any delay in presentation to the hospital and the presence of any other contraindication, means that most patients (around 95%) do not receive the drug. Second, many patients do not reperfuse even when given the drug, and up to 5% will experience haemorrhagic complications as the result of the drug administration [10].

Neuroprotection is an entirely different approach. In general, as stated above, the role of the neuroprotective agent is to interfere with one or more of the mechanisms involved in the 'ischaemic cascade' and thereby limit the resultant tissue damage. The assumption is that the area of very reduced blood flow (the ischaemic core) is surrounded by the penumbra, which is compromised by the low blood flow, but can be protected either by reflow, or by administration of the neuroprotectant. Without such intervention the cells in the penumbra will also die and the core will expand (Figure 3). There is much evidence available to support this view [11]. Without intervention the ischaemic cascade will also encompass the other mechanisms that lead to cell death (Figure 2).

Animal models of stroke

There are several models of acute ischaemic stroke available and investigators have often introduced modest personal modifications to the main models [12,13]. There is insufficient space here to critically review the various models but it is reasonable to state unequivocally that the most relevant models involve an occlusion of the middle cerebral artery (MCA) because the majority of human strokes result from an occlusion of this artery [14]. Transient MCA occlusion (MCAO) mimics the problem of both ischaemia and reperfusion, whereas permanent MCAO models the problem of long term vessel blockade, as often occurs in humans [15]. Haemorrhagic stroke models are also available and have been used to examine the consequences of a bleed in the brain [16].

While most people involved in any aspect of drug discovery would emphasize the value of appropriate animal models, this has been a contentious area in stroke research. A review of some of the reasons why animal models are useful in the development of drugs to treat acute ischaemic stroke was recently published [17]. However, this seems to have done little to settle matters and this journal (*Drug Discovery Today*) recently published an article in

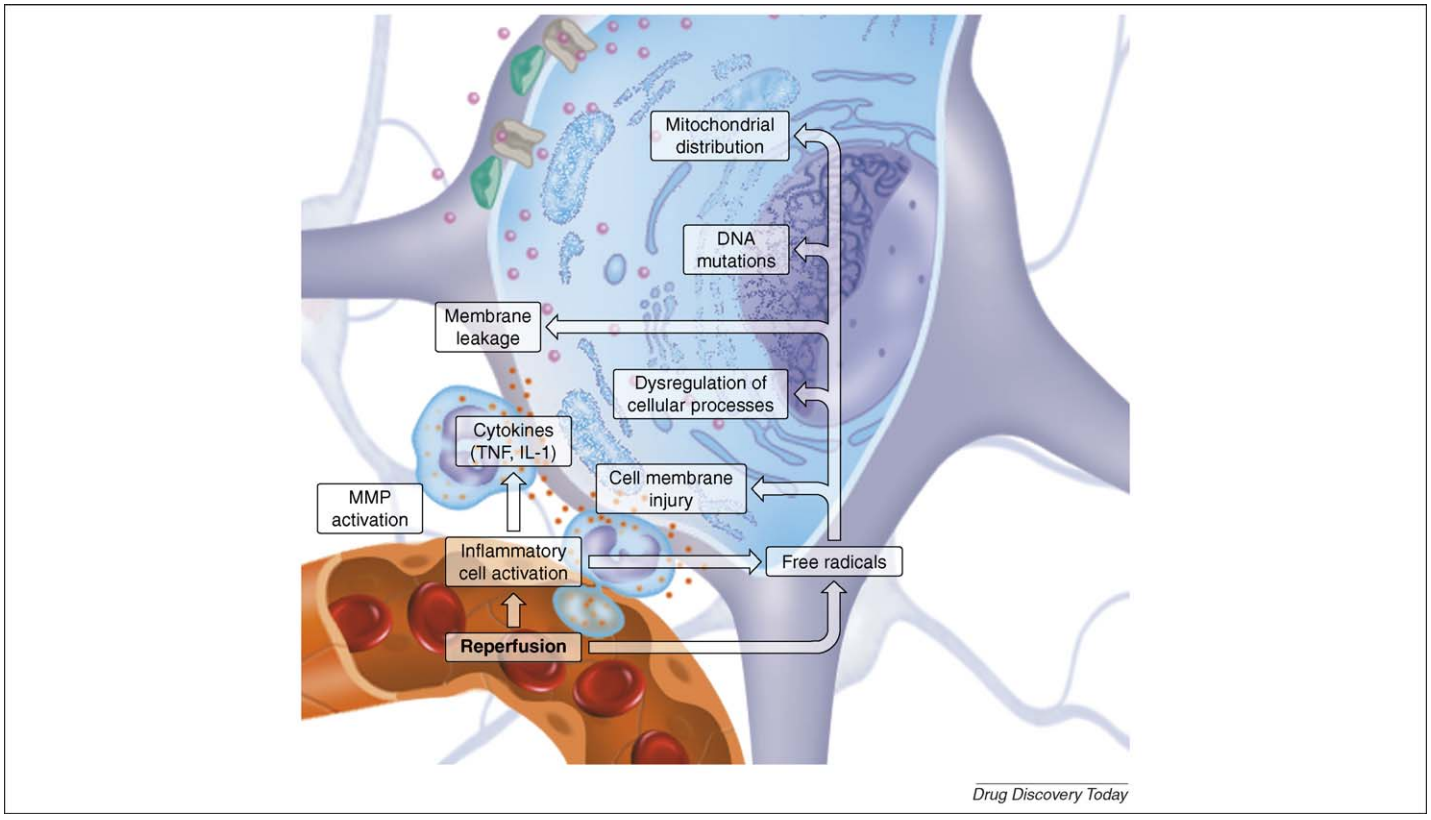

FIGURE 2

Major biochemical pathways involved in cell damage following reperfusion. If reperfusion occurs soon after occlusion then only some of the earlier changes down the pathways might occur. These neurodegenerative mechanisms will also occur following sustained ischaemia because of the production of free radicals and damage to the endothelial cell wall.

which the statement occurred that: 'For stroke, the [animal] models and the clinical condition are extremely different, which is reflected in the failure of molecules in the human condition' [18]. The author ignored many other more persuasive reasons for explaining the failure. Nevertheless, it is a view that also surfaced at the same time elsewhere [19] and was then discussed in an abbreviated way [20,21], which means that it is probably a widely held view. Why? Partly, of course, because the failure so far of compounds to make the successful transition from efficacy in animal models to the clinic has meant that we cannot yet ascertain whether any one model is appropriate for predicting the clinical value of an experimental drug. However, there are more compelling reasons for scepticism. Animal models almost invariably use young healthy animals, whereas stoke patients are usually elderly, with a variety of other clinical problems, such as hypertension, myocardial infarction and diabetes, and generally none of these problems is included in the animal model. Nevertheless there is good evidence that many of the physiological factors that influence ischaemic damage in animal models also have the same effect on stroke outcome in patients, which does indicate cross-species validity [17]. It is also notable that focal ischaemia in marmosets induces not only motor problems in the contralateral arm, but also spatial hemineglect, and both of these problems have exact clinical correlates [22,23].

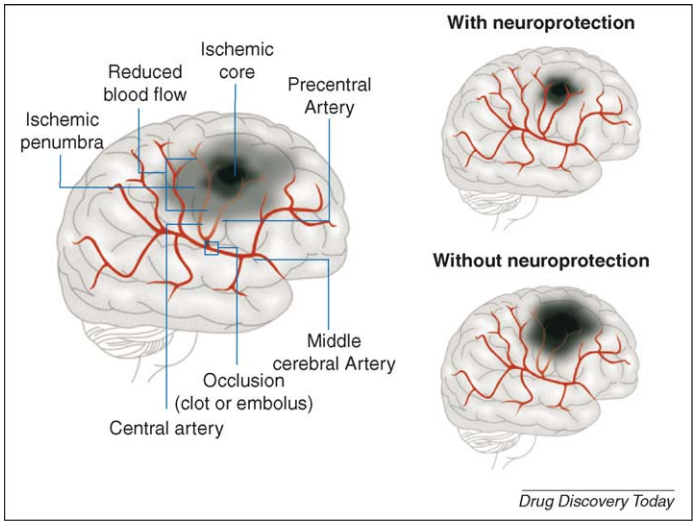

FIGURE 3

An occlusion of a branch of the middle cerebral artery. The middle cerebral artery has an indication of the ischaemic core area and the penumbra. The figure shows the spread of damage as occurs with and without neuroprotective drug administration.

We should therefore find reasons, other than poor animal models, for the clinical failure of the experimental compounds developed to date. What can be argued is that most negative results resulted from the failure of scientists to apply the information supplied by animal models to the clinical trial. For example, the *N*-methyl-D-aspartate (NMDA) antagonists were only found to be neuroprotective when given to rats up to 90 min after occlusion

[24,25], but for the vast majority of patients in the clinical trials these drugs were not given until ~6 h later. In many cases the drug exposure in patients has been only a fraction of the dose required to provide the best achievable neuroprotection in rodents, primarily because of adverse events when the drugs were given to humans at doses that would produce similar exposure to that required in rats. The view had been that the time window of opportunity in humans would be longer than in rats and that the drug exposure could similarly be compromised. Therefore, drugs were never given to humans using conditions required for maximum benefit in the animal models. Furthermore, although there is good evidence that most patients reperfuse slowly [15], several drugs were examined clinically as monotherapy despite the fact that were only effective in reperfusion models and would therefore only be expected to work when given with a thrombolytic.

We can also suggest that clinical trials have also been badly designed, not only by failing to take into account available information from the preclinical investigations but also by using small patient numbers because of assumptions of unrealistically large benefits, inappropriate patient selection, outcome measures and data analysis [26].

Stroke Therapy Academic Industrial Roundtable criteria

The ever-increasing number of drug failures in the clinic during the late 1990s resulted in a meeting of experts from academia and industry to formulate guidelines on what information had to be collected in animal models before a drug could be considered for progression to clinical trial in an attempt to maximize the chance of success. The group was called the Stroke Therapy Academic Industrial Roundtable (STAIR) and the guidelines in their resulting publication [27] are now often referred to as the STAIR criteria. Recently, we published modest additions [17] in the light of other information gained (Table 1). Some of the proposals on the list might, with the benefit of hindsight, seem obvious, and some are certainly standard pharmacological rules that should be applied to any drug discovery research (dose–response data for example). Nevertheless it is worth reminding ourselves that several of these guidelines were formulated as the result of information gained in the earlier failed studies. NXY-059 is the first neuroprotective drug developed using strict adherence to the STAIR criteria before entering Phase III [28] and this drug has recently produced a statistically significant improvement in stroke patients (using the modified Rankin global disability score for assessment) [29] (Box 1).

In conclusion we would point out that the STAIR criteria emphasize the view of many experts in industry and academia that animal models will continue to play a vital role in the drug discovery process in the future and that we concur with that opinion.

BOX 1

Outline of the recommendations of the Stroke Therapy Academic Industry Roundtable [27] as modified by Green *et al.* [17] that should be met before a compound is progressed to clinical trial.

STAIR recommendations

- Adequate dose–response plus serum concentrations measured, thereby defining minimally and maximally effective doses
- Time window studies confirming efficacy. Time and duration of drug administration appropriate to the mechanism of action and appropriate to the proposed clinical protocol
- Physiological monitoring of animals undertaken
- Randomized, blinded studies; reproducible effects (one independent)
- Infarct volume measured, compound provides sub-cortical as well as cortical protection.
- Histological protection of >70% in both transient and permanent focal ischaemia when drug is given 15–30 min after occlusion
- Attenuates white matter damage in brain
- Functional tests used, short and long term assessment
- Small rodent studied with permanent middle cerebral artery occlusion (MCAO). Must show efficacy in permanent MCAO models; compound is efficacious as monotherapy
- Larger species used for novel, first-in-class compound
- Studies published in peer-reviewed journal

Thrombolytics and anti-aggregation compounds

Thrombolysis

Alteplase (rt-PA) is licensed and in clinical use for acute ischaemic stroke and will therefore only be considered briefly. rt-PA was licensed on the basis of two positive Phase III clinical trials, using a 3 h time window [9]. Crucially for the discussion on animal models above, this compound is effective in a rat thromboembolic stroke model and, in that model, it is effective up to 3 h after the infarct [30]. Time window studies are continuing in patients, but completed negative trials using longer time windows have already been reported [31]. The compound increases haemorrhagic transformation in animals [32,33] and stroke patients [10], associated with the drug increasing the levels of matrix metalloproteinase-9 (MMP-9, see Figure 2), which thereby increases haemorrhage rate [34,35]. Recently it has been suggested that if rt-PA reaches the extracellular space it could be neurotoxic [36], although it remains unclear whether this reflects a possible clinical problem.

Desmoteplase is a new thrombolytic now being developed for use in stroke. It is a genetically engineered version of a thrombolytic protein that is present in the saliva of the vampire bat [37]. Because desmoteplase has a strict requirement for a fibrin cofactor, its activity is considerably enhanced, compared to rt-PA, in the presence of fibrin [38]. It has therefore been proposed that desmoteplase will dissolve blood clots without altering systemic clotting, thereby reducing the rate of haemorrhage compared to rt-PA. Although available preclinical data have so far failed to support this contention [39], such problems could be minimized by using the appropriate dose and time of administration in patients [40]. Studies on its effect on neurodegeneration have also been performed and it has been suggested that it has less potential than rt-PA to cause neurotoxicity [41,42].

The first Phase II trial of desmoteplase examined the compound in patients with magnetic resonance imaging (MRI) evidence of perfusion–diffusion mismatch at enrolment using a 9 h therapeutic window. The first part of the study was terminated because of excessive rates of intracranial haemorrhage. The second part of the study used a lower weight-adjusted dose (up to 125 μg/kg). The 15 patients in the top-dose group had a higher rate of early reperfusion, blood flow and no increase in symptomatic haemorrhage rate

when compared with the placebo group [40]. A Phase III trial with penumbral imaging is currently running.

Anti-aggregation compounds

Abciximab is a monoclonal antibody that binds to the glycoprotein IIb and IIIa receptor on the surface of platelets, promotes fibrinolysis – it inhibits clot formation by preventing the platelets from sticking together [43]. It is in clinical use to restore coronary blood flow, often in combination with thrombolytics. Recently there has been interest in the possible role for this compound as a reperfusion agent in stroke. Because abciximab is not active in rodents [44], a close analogue [7E3 F(ab′)$_2$] has been investigated in rat stroke models. In a rat focal thromboembolic model Shuaib *et al.* [45] showed that both rt-PA (10 or 20 mg/kg) and 7E3 F(ab′)$_2$ (6 mg/kg) reduced infarct size, as did the two drugs in combination. There was an increase in haemorrhage rate with both drugs and the highest incidence of haemorrhage occurred in a group given the high dose combination. Somewhat different results were obtained by Zhang *et al.* [46], who found a decrease in infarct size in rats treated with a 7E3 F(ab′)$_2$–rt-PA combination but no reduction in infarct size when the drugs were administered individually. However, this apparent difference might result from the later time of administration than that used in the Shuaib *et al.* study [45]. In a subsequent study it was found that giving 7E3 F(ab′)$_2$ to rats also administered tenectaplase resulted in a decrease in infarct size when both were given at 4 h [47].

A recent company press release announced the termination of the Phase III trial of abciximab in stroke, following a recommendation by the independent safety and efficacy monitoring committee.

Other perfusion enhancing approaches

Other approaches have been examined clinically to increase blood flow in the brain of stroke patients, including intravenous administration of the defibrinogenating compound ancrod. A Phase III trial in which ancrod was given within 3 h of stroke onset to 500 patients produced a favourable response compared to placebo, but with an increase in cerebral haemorrhage rate [48]. A Phase III trial of the compound given up to 6 h after stroke onset was negative, but a new trial using this time window was initiated in September 2005.

Intra-arterial thrombolysis with prourokinase was investigated [49], but does not appear to be being developed into routine use. Aspirin might confer modest benefit if given within 48 h of stroke [50] and heparin has also recently been suggested to confer possible benefit if given within 3 h, although an increase in cerebral haemorrhage was seen [51].

Neuroprotective agents

The major cause of the pessimism about the value of pharmaceutical intervention to treat acute ischaemic stroke has been the results obtained with neuroprotective agents. Thrombolytics can at least claim limited success. Neuroprotectant approaches have yet to claim unequivocal evidence of success, although one compound recently produced clear evidence of efficacy in a Phase III trial (NXY-059; see later).

Many compounds that have been clinically examined have belonged to only one class, the glutamate antagonists [predominantly NMDA receptor subtype antagonists, but also some α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists and a glycine modulator site antagonist]. However, there have also been a substantial number of compounds examined that have acted at many other sites on the ischaemic cascade (Table 1). These failures have resulted in many companies withdrawing from the race to develop an effective neuroprotectant. Indeed, even over the last few months further failures have been reported, some of which will be briefly reviewed below because they are likely to influence future drug discovery programmes. Although there are a limited number of new compounds for us to review compared with ten years ago, there continue to be new approaches being developed that will be examined here in some depth where possible.

Drugs altering glutamate function

Despite the consistent failure of NMDA antagonists to exhibit any sign of clinical utility, some compounds with glutamate antagonist activity still appear to be in development, although it remains unclear how active the development efforts are. In our view the problem with glutamate antagonism as a therapeutic target is the fact that pathological glutamate release is an event very early on in the ischaemic cascade (Figure 1), which makes it necessary to give such drugs very quickly after the ischaemic insult [24,25,52], a significant problem in the clinical situation. Other problems observed with some of these drugs included poor pharmacokinetics, brain penetration and a failure to protect subcortical structures.

There appears to be only one NMDA antagonist compound currently being developed. This is traxoprodil (CP-101,606) which is an NMDA antagonist with NR2B receptor subtype selectivity [53]. It was developed following increased understanding of the NMDA receptor subtype pharmacology and evidence gained about earlier compounds with NR2B subtype selectivity, such as ifenprodil, eliprodil and felbamate [54]. There is evidence that it acts as an effective glutamate antagonist *in vitro* using a primary cortical neurone preparation [55], but information on its action *in vivo* is very limited. It has been shown to reduce damage after cortical compression-induced brain ischaemia [56] and when given 15 min before MCA occlusion in the cat [57]. More crucially, the compound was effective as a neuroprotectant when given 2 h after clot insertion in a rat thromboembolic stroke model, displaying a dose-dependent decrease in infarct volume [58]. The lack of information about this compound from the company might indicate that it is seen as a high-risk project and is not being pursued with high activity.

It has long been known that reducing the magnesium concentration *in vitro* enhances NMDA-induced electrophysiological responses; that is, Mg^{2+} 'gates' the NMDA receptor channel [59]. Magnesium also reduces presynaptic glutamate release [60]. Studies of stroke in animal models found that a dose of magnesium sulphate in transient [61,62] and permanent [63] MCAO models was effective in producing neuroprotection. Yang *et al.* [64] examined the time window in an embolic stroke models and found a statistically significant effect of magnesium at 6 h but not 8 h. These, and other similar data, encouraged small pilot studies on the effect of magnesium in stroke patients that provided sufficiently encouraging results to initiate a full clinical multicentre

trial. This examined almost 2400 patients and although detecting no reduction in death or disability it did note a possible benefit in lacunar strokes [65]. However, this was the opposite of what was hypothesized in the trial.

The possible value of antagonizing the action of the NMDA receptor by acting at its glycine regulatory site [66] has also been pursued vigorously, and has resulted in the development of gavestinel. The failure of the compound in a Phase III trial [67] has probably terminated any further interest in this pharmacological approach.

AMPA antagonists have also received significant interest as neuroprotectants. However the prototypic competitive antagonist 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(f)quinoxaline (NBQX) suffered from low solubility problems and produced kidney toxicity. Subsequent compounds produced have been competitive and non-competitive, but the competitive antagonists have the propensity to induce sedation, visual disturbance and memory impairment [68]. The development of YM 872, the only AMPA antagonist to have been in recent clinical investigation, has been terminated.

There has also been some interest in glutamate transporters as drug targets for stroke and, although this is of theoretical interest, this investigative line does not seem to have been followed up seriously in terms of drug development [69].

5-hydroxytryptamine 1A agonists

Repinotan (Bay x3702) is a potent 5-hydroxytryptamine 1A (5-HT_{1A}) receptor agonist. Such compounds have been known for many years to produce hypothermia in rodents [70], but there are no indications that this is its mode of action as a neuroprotectant. Rather, it is proposed that it acts by inhibiting neuronal firing in the dorsal raphé nuclei, which leads to the inhibition of excessive ischaemia-induced glutamate release. It might also produce hyperpolarization, thereby reducing anoxia-induced depolarization.

It is difficult to fully assess the profile of repinotan in animal models of stroke because of limited published data. Much of the evidence is contained in a single publication where it was reported that the compound was effective in transient and permanent focal ischaemia resulting from MCA occlusion [71]. In these studies repinotan produced almost total neuroprotection (as measured by infarct size reduction compared with saline-injected control animals) when a dose of 10 μg/kg/h infusion was given for 4 h immediately after the start of a 1 h transient occlusion and an 81% decrease when given 5 h later. In a permanent ischaemia model the same dose of repinotan given immediately after the occlusion reduced infarct size by 65% and by 43% when treatment was initiated 5 h post-occlusion. Plasma levels were not measured so it is difficult to compare the doses with the clinical dosing schedule used (1.25 mg/day for 72 h) calculations on dosing suggest approximately half the dose being given to humans than that often used in rats.

The initial Phase III clinical trial was initiated and then modified and reclassified as a Phase IIb study with an inclusion time window of 4.5 h. A decision was made by the company to terminate development in December 2004 because of the failure of the drug to meet efficacy endpoints.

Piclozotan (SUN N4057) is another potent 5-HT_{1A} agonist now in clinical development (Phase IIb) for stroke. However apart from evidence that it is active when given immediately following the start of a transient MCA occlusion [72], no data are available to allow assessment as to whether this compound offers properties to differentiate it from repinotan.

Metal chelation

Metal ions are vital in controlling for enzymes, cofactors and cellular transporters, including several whose disruption have been proposed to be intimately associated with cell death following acute cerebral ischaemia. These include MMPs, calpain and Cu–Zn superoxide dismutase. Disruption in metal ion homeostasis has been suggested to be involved in various chronic neurodegenerative conditions such as Parkinson's and Alzheimer's diseases [73]. The probability that disturbance in metal ion regulation could also be associated with cerebral ischaemia (as indicated by the evidence that disturbances in zinc homeostasis was associated with cerebral cell death following transient focal ischaemia [74]) has resulted in one recent novel approach to drug development. DB-b99 is a derivative of 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), a compound that chelates divalent metal ions including zinc, calcium, iron and copper [73]. This action could explain its beneficial effect in inhibiting the oxidative-stress-induced increases in calpain *in vitro* [75] and ischaemia-induced MMP activation in the brain of rats subjected to MCA occlusion [76]. The compound is reported to decrease infarct size in a rat MCA occlusion model [76] but, because the only data are in abstracts, it is impossible to evaluate the work critically. Nevertheless the compound is now reported to be in Phase II trials and appears to be well-tolerated in healthy volunteers [77].

Another metal chelator that decreases infarct volume in a rat transient focal ischaemia model is PAN-811. This was originally developed as an anticancer drug because of its ability to chelate iron, but also found to be able to modulate calcium homeostasis and therefore presumably reduce free radical production [78]. This compound decreased infarct size by a modest 35% in a transient MCA occlusion model and also had a narrow dose window [78], which argues against clinical development. However these data also indicate the probable value of metal chelation as a mechanistic approach to neuroprotection.

Citicoline

Citicoline is cytidine-5′diphosphocholine (CDP-choline), a compound that has been in clinical use for many years in Europe and Japan for a variety of degenerative neurological disorders. It is an intermediate in the biosynthesis of phosphotidylcholine, which is of key importance in regulating cell membrane integrity. Phosphotidylcholine is broken down to free fatty acids during ischaemia, which generates free radicals that induce further cell damage [79]. Citicoline, by reducing lipid metabolism following ischaemia, thereby presumably reduces the levels of free fatty acids [80,81] and therefore free radical production [82].

Studies on the effects of citicoline on ischaemic damage in experimental animals go back several years and there is good evidence for its efficacy in several models of acute ischaemic stroke. Such studies include reduction of neurological deficits in a rat global ischaemia model [83], decreased infarct size in gerbils subjected to transient forebrain ischaemia, and transient focal ischaemia in rats [84]. Citicoline was also effective in significantly reducing infarct size in a rat thromboembolic focal ischaemia

model [85]. Variations in methodology prevent meaningful comparison of doses and therapeutic window data.

Four Phase III studies have been conducted on citicoline, all with fairly small patient numbers and with various doses (500–2000 mg) being administered to patients with variable stroke severity. The trials showed trends for improvement but any conclusions were compromised by either small cohort size or possible inappropriate primary outcome measures being employed. Dávalos *et al.* [86] therefore performed a meta-analysis on all data generated from the 4 trials to assess the efficacy of citicoline. Results from 1372 patients (583 placebo and 789 citicoline) suggested a beneficial effect of the drug ($p = 0.034$) with the greatest effect at the highest dose. A further Phase III trial is now being planned.

Arundic acid

Arundic acid (ONO-2506) is an astrocyte-modulating compound that inhibits the synthesis of the protein S-100β in cultured astrocytes and has been shown to inhibit the increase in the concentration of S-100β in the cerebrospinal fluid and plasma of rats subjected to transient or permanent MCAO [87]. S-100β has been implicated in producing cell death through its activation of several intracellular signalling pathways [88]. The compound has a wide range of actions in cultured astrocyte preparations including actions on γ-aminobutyric acid (GABA) A receptors, glutamate transporters and lipopolysaccharide-inducible nitric oxide synthase expression [88].

In studies on the affect of arundic acid in animal models of stroke it has been found to have a therapeutic time window that is very long, 24 h in transient MCAO and 48 h in permanent MCAO, which has been ascribed to its effects on S100β and to the other mechanisms outlined above [88]. Other studies, including one on primates that suggested a positive effect of the drug in animals subjected to permanent MCAO, have only been reported in abstract form and are reviewed by Asano *et al.* [88].

In May 2005 the company developing the compound (Ono) reported that the Phase II clinical study of the drug for acute stroke initiated in the USA and Canada would be terminated following a futility analysis by an independent board of advisors. Results on this study and a study that is continuing in Japan have yet to be made available.

Free-radical scavengers and trapping agents

There is compelling evidence to support the notion that free radicals have a significant role in the causation of cerebral tissue damage following both ischaemia and reperfusion [89,90]. Consequently, several compounds have been developed that are designed to remove free radicals and thereby lessen damage.

Ebselen is a selenium compound that possesses glutathione-peroxidase-like activity and might therefore act as a mimic for this enzyme rather than being a free-radical scavenger [91]. There is evidence that the compound is protective in transient ischaemia, but a recent study showed it to be ineffective in the rat permanent MCA occlusion model [92]. Only small clinical studies have been performed and these did not provide clear evidence for efficacy in stroke so development was terminated. Both preclinical and clinical studies on ebselen were recently reviewed elsewhere [28].

The lazaroid compound tirilazad possesses free-radical scavenging activity and has been examined extensively in both animal models of cerebral ischaemia and in stroke patients. However, it is ineffective in rat permanent ischaemia models [93] and there is no evidence for its efficacy in transient ischaemia models when given several hours after the insult. Tirilazad would not therefore now be considered seriously as a candidate drug for clinical development because of its failure to meet several of the STAIR criteria. The negative outcome in several clinical trials cannot be claimed to be unexpected in the light of current knowledge [28].

Edaravone is a hydroxyl radical scavenger that has been examined in a variety of experimental disease models, including stroke. There is limited evidence for efficacy in stroke models but nothing published on dose–response data, and most studies gave the compound almost immediately after the ischaemic insult. The compound also failed to protect subcortical structures [28]. Clinical data are sparse, and although this compound has been approved by the regulatory authority in Japan to treat stroke patients this appears to be on the basis of a single placebo-controlled study in a small number of patients. The developmental status of the compound outside Japan is unknown.

NXY-059 is a novel neuroprotectant with free-radical-trapping properties [52,94] and is now in Phase III clinical development for acute ischaemic stroke. NXY-059 is a nitrone and is the first compound to have been developed in accordance with the STAIR criteria and indeed meets all the suggested criteria required for clinical investigation. The compound has been shown to produce clear dose-dependent neuroprotection in rats in transient and permanent MCA occlusion models of ischaemia, including subcortical protection [5]. NXY-059 also has a wide window of opportunity producing statistically significant neuroprotection when given at 4 h after permanent focal ischaemia [5] and 5 h after transient ischaemia [95]. In marmosets it lessened the motor deficits in the paretic arm and also spatial hemineglect, even when given 4 h after permanent MCA occlusion [23,96]. Thus the compound provided clear evidence for producing functional improvement in the use of the paretic arm following experimental stroke (Figure 4) in

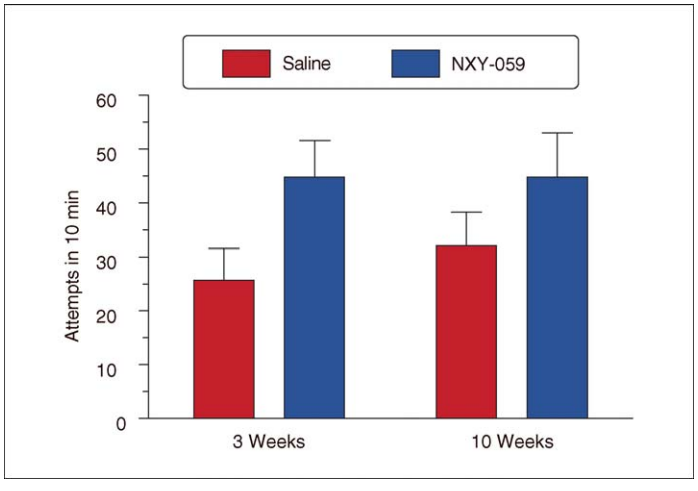

FIGURE 4

Use of a paretic arm to retrieve food rewards in marmosets administered either saline or NXY-059. Saline or NXY-059 were administered 4 h after permanent middle cerebral artery occlusion, and measurements of food-retrieval by the marmosets were taken 3 and 10 weeks later. Data taken, and redrawn, from Ref. [96].

addition to evidence for it decreasing infarct size using histological techniques.

The evidence for NXY-059 meeting all the STAIR criteria has been reviewed elsewhere [28] but it is worth emphasizing that the window of therapeutic opportunity data (greater than 4 h) and the evidence that the compound has a maximal effective neuroprotection in rats at a plasma unbound concentration of 24 μmol/l in transient focal ischaemia and 140 μmol/l in permanent focal ischaemia models, allowed direct translation to the design of the clinical trial. NXY-059 had been shown to be well-tolerated in stroke patients at a plasma unbound concentration of 260 μmol/l [97]. Consequently, Phase III trials could be designed where the plasma concentration of drug in the patients exceeded the concentration known to be maximally effective in rodent models and the time window of inclusion matched that known to be effective in both rodent and primate models. This is the first time that this has been achieved [28].

Confidence in the correctness of this drug development approach to the treatment of acute ischaemic stroke has now been increased by the analysis of the first of the two Phase III trials of NXY-059 in acute stroke patients (the so-called SAINT I trial) because the drug was found to significantly reduce disability (using the modified Rankin score) when administered within 6 h of stroke onset without apparent tolerability or safety issues [29]. NXY-059 did not improve neurological function as measured by the US National Institutes of Health stroke scale [29]. Additional data to confirm the benefit of this drug in stroke patients are now awaited from the companion SAINT II study.

It remains unclear why NXY-059 has such a superior profile to the free-radical scavengers reviewed above in preclinical stroke models and in clinical trial. NXY-059 has been termed a free radical trapping compound (rather than scavenger) because it produces a relatively stable adduct, and thus its radical trapping ability might be rather less reversible than some other compounds. It is also worth mentioning that NXY-059 prevents mitochondrial dysfunction after an ischaemic insult because it attenuates ischaemia-induced cytochrome C release and maintains Akt activation in ischaemic brain tissue [98,99]. The drug might therefore modify biochemical pathways that lead to cell death or survival (Figure 5).

Recently data have been published on the effect of another nitrone-derived compound, stilbazulenyl nitrone (STAZN), in transient MCAO. These indicate that STAZN can also produce effective neuroprotection in this model [100]. Although this compound might have modestly greater penetration of cerebral tissue [101] than NXY-059 [102], the importance of this property remains unclear because an action in the microvasculature of the neurovascular unit could be sufficient for effective neuroprotection [103].

Biopharmaceuticals

Several approaches to the treatment of stroke are now being evaluated that involve either biopharmaceuticals or initial biopharmaceutical studies with the hope that a small molecule approach could be developed from the data obtained.

There is a considerable body of information on the importance of cytokines, particularly tumour necrosis factor (TNF) α and

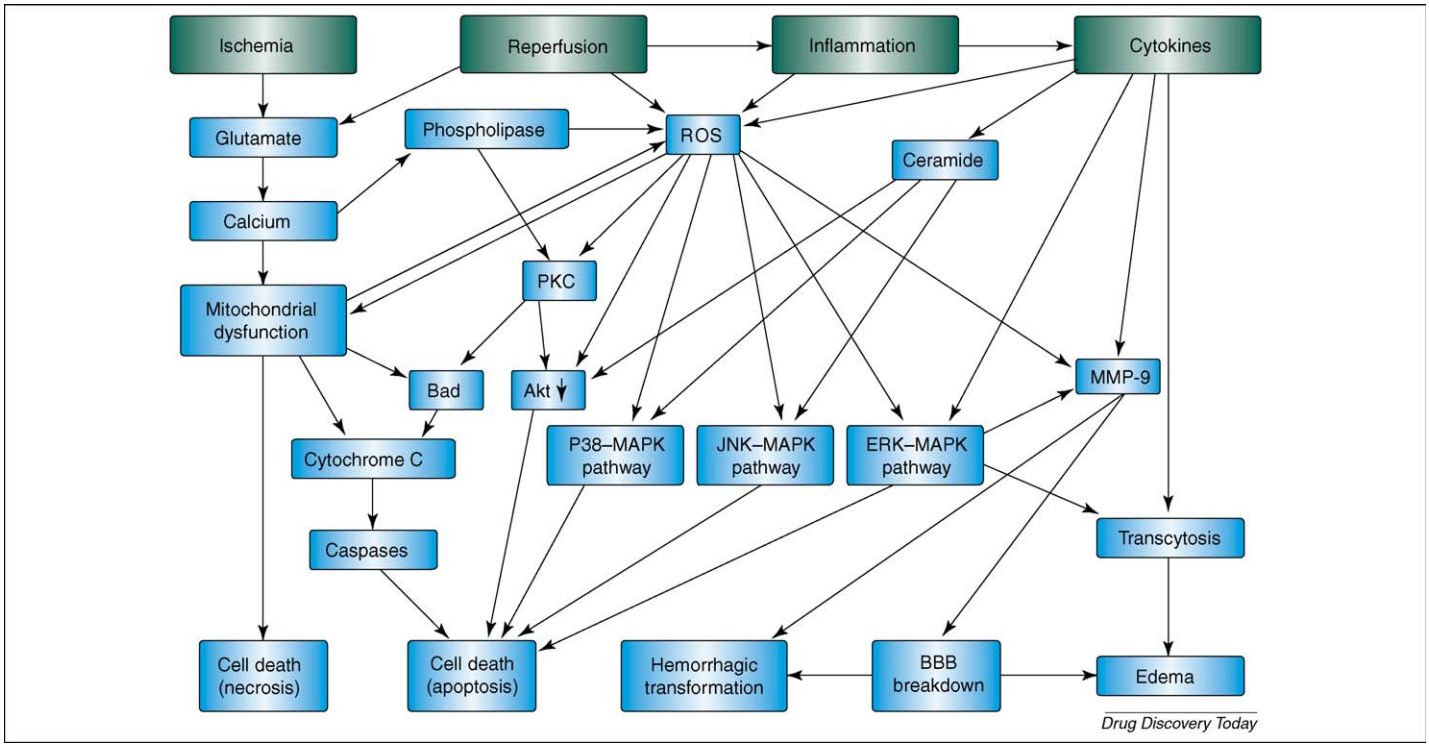

FIGURE 5

Some of the pathways proposed to be involved in cell death and cell survival mechanisms following cerebral ischaemia. This figure is shown primarily to illustrate possible future targets for drug therapy. It is simplified and illustrates only major pathways, so it does not show that some pathways can lead to either cell death or cell survival depending on the severity or duration of the ischaemia and that there remains discord among investigators as to the specific role of several of the signalling systems.

interleukin (IL) 1β (Figure 2), in the inflammatory response of the brain to injury [104,105]. Furthermore, TNFα antibodies [106] and IL-1β antibodies [107] have been shown to inhibit ischaemia-induced damage to the brain. However, the action of TNFα in stroke is complicated because there is evidence that it can also assist cell survival [108,109]. A recombinant IL-1 receptor antagonist has recently been found to be well tolerated in a Phase II study in stroke patients [110].

Although protein-based anti-TNFα compounds are available, efforts are now being made to produce non-peptide compounds. These were recently reviewed by Lovering and Zhang [111] and include targeting enzymes involved in the biosynthesis of TNFα, such as the mitogen-activated protein kinases. The other approach is to produce a compound that will act on TNFα-converting enzyme (TACE), a protease that generates soluble TNFα from the membrane-bound form. There is evidence that some compounds with known TACE inhibitory activity are neuroprotective in animal stroke models, but the fact that these compounds also inhibit matrix metalloproteinases (key enzymes involved in blood–brain barrier damage) currently provides problems in clarifying whether TACE inhibition will provide a new therapeutic approach to stroke.

Other approaches to stroke therapy involving growth factors have also been recently reviewed [112]. These include basic fibroblast growth factor (bFGF), osteogenic protein-1 (OP-1), vascular endothelial growth factor (VEGF), erythropoeitin (EPO) and granulocyte colony stimulating factor (GCSF). All of these compounds have been examined in animal stroke models and some (bFGF and EPO) have progressed to initial clinical trial. Although it is suggested that such compounds might have more applicability in assisting long term recovery rather than acute treatment [112], some, for example, FGF, also have neuroprotective effects if given soon after an acute MCAO [113].

Albumin administration has also been demonstrated to produce neuroprotection, decreasing infarct size in rats subjected to transient [114] and permanent [115] MCAO; in the transient model it has a therapeutic window of 4 h after occlusion [116]. There is evidence that part of its effect is from haemodilution [114], but other mechanisms have also been invoked to explain its neuroprotective action [114,116]. A small open study has been conducted on the effect of albumin given to patients within 24 h of stroke onset and this suggested increased cardiopulmonary adverse events [117]. By contrast, a small Phase I trial, did suggest albumin to be well tolerated [118] and a Phase II/III study is now beginning enrolment.

Thrombolysis and neuroprotection?

The combination of a thrombolytic and a neuroprotectant could offer advantages like synergy in the degree of clinical improvement produced, in extending the treatment window for rt-PA, in decreasing the incidence of problems (such as the thrombolytic-induced increase in haemorrhage rate), or in a combination of all these factors [119].

Demonstrating the value of combination treatments (e.g. proving synergy rather than mere addition) is much more complex – even in animals – than is perhaps realized, but proposals for factorial designs have now been published [120]. Several studies have been reported recently, including a study of the NMDA antagonist traxoprodil with rt-PA, which suggested no enhancement of the neuroprotective effect of traxoprodil [58], and NXY-059 with rt-PA, which found that the combination did not confer additional benefit than that seen with NXY-059 alone [121]. Both these studies, and others, failed to unequivocally indicate that a thrombolytic combined with a neuroprotective has a synergistic effect. However, another study [122] did find that NXY-059 could prevent the increase in haemorrhage rate produced by rt-PA, which is in agreement with studies by this group and others [33] on other nitrones. Importantly the recently reported analysis of the SAINT 1 clinical trial on NXY-059 indicated a treatment benefit irrespective of whether or not the drug had also been given with rt-PA and also observed that the group receiving rt-PA with NXY-059 had fewer haemorrhages than those receiving rt-PA with placebo [29]. Thus two major observations made in experimental animals were also seen in the subsequent clinical trial.

Conclusions

The continuing failure of compounds developed for the treatment of acute ischaemic stroke has meant that relatively few compounds are now in late development phase. Furthermore, given the failure of related compounds, some other approaches in early development are now looking increasingly unlikely to reward further efforts. At present only two neuroprotective compounds appear to have a chance of success in the near future: Citicoline (primarily because of analysis of the trials suggest it might not have previously demonstrated efficacy because of poor trial design); and NXY-059 (which is the first neuroprotective agent to have produced clear statistically significant improvement in a global rating scale following a Phase III trial). The results of the second Phase III trial are thus eagerly awaited. The plausibility of a second positive trial is raised by its preclinical development having been conducted in accordance with the STAIR criteria, as well as the alignment of the design to the preclinical data when deciding the plasma concentration to be targeted in patients and the time window of administration.

Restoration of cerebral perfusion with rt-PA has a proven track record of success in a small defined group of patients. New compounds, such as desmoteplase and ancrod, might in time also demonstrate success. However, the problem of haemorrhagic transformation might also be seen with these compounds, which would produce be substantial limitations on their clinical use.

It is likely that there will be an increase in research to develop compounds that act to modify the cell survival and/or cell death pathways (Figure 5). This is a complex area – the action of some of the factors shown in Figure 5 can lead to cell survival or cell death depending on the duration or severity of the insult or the isoform being targeted [123]. There even remains disagreement among those working actively in this area as to the specific role of some of the pathways shown [124]. What is clear is that there are complex interactions ('cross-talk') that exist between these pathways. Consequently, although there remain various experimental approaches being pursued, little is known about the *in vivo* ramifications of such interventions, let alone any published data indicating whether compounds targeting the pathways will meet the STAIR guidelines.

The importance of an adequate understanding of the safety and tolerability profile of all future compounds, as well as the need to

set realistic expectations on benefit in accordance with results to date, implies that large trials will be required to satisfactorily address risk and benefit and bring forward new treatment options that this therapeutic area so needs. It is hard to believe, in the light of many failures, that companies will be willing to fund large trials without reassurance of data addressing the STAIR requirements, particularly in view of their apparent value in the so-far successful development of NXY-059.

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