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Failure in developing effective treatment for cerebral ischemia: The stair recommendations

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ABSTRACT

Cerebral ischemia leads to neurological dysfunction and activate complex signalling cascades, which may depreciate survival mechanisms as a result of cellular homeostatic breakdown. For acute ischaemic stroke, safe and effective treatments that offer an extended therapeutic window are urgently needed to decrease disability and aid neurological recovery. The benefit of recombinant tissue-type plasminogen activator (rt-PA) when initiated within 3 hours of stroke onset documents that acute ischemic stroke can indeed respond to treatment. This news is countered by the current lack of documentation that any purported neuroprotective drug significantly improves outcome when given after stroke onset. Lessons have been learned from the innumerable unsuccessful thrombolytic and neuroprotective trials, suggesting that future, better designed trials will likely demonstrate significant benefits with appropriate safe and effective drug treatments initiated within 6 hours of stroke onset. Also, the age of combination drug trials is approaching and combination treatments directed at both the vascular and cellular mechanisms of ischemic brain injury are likely to have the greatest impact upon stroke disability. The apparent failure of neuroprotection for acute ischemic stroke has caused a series of active discussions in both academic and industrial fields, which is fruitful and constructive in attempts to establish criteria for preclinical stroke studies. Since the Stroke Therapy Academic Industry Roundtable (STAIR) published its first criteria in 1999 and updated, quality of study design problems and inflation of reported efficacy of neuroprotectants have continued to be major issues in this field. Translational research of neuroprotection for ischemic stroke has reached its critical stage. A strategic reconsideration is urgently needed to aid in the search for new solutions.

KEYWORDS

Cerebral ischemia, recombinant tissue-type plasminogen activator, neuroprotection, STAIR criteria.

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1. INTRODUCTION

Cerebral ischemia is a condition in which there is inadequate blood flow to the brain to meet metabolic demand leading to reduced oxygen supply or cerebral hypoxia and thus causing the death of brain tissue or ischemic stroke. It is a sub-type of stroke besides subarachnoid hemorrhage and intracerebral hemorrhage. Of all strokes, 87% are ischemic, 10% are intracerebral hemorrhage, and 3% are subarachnoid hemorrhage. According to WHO, stroke was the second commonest cause of worldwide mortality in the 1990s and, the third commonest cause of mortality in developed countries. It is also majorly associated with long-term disability and, has enormous emotional and socioeconomic consequences for patients, their families, and health services. The case-fatality rate due to stroke is reported to vary varies from 10% to 35%.[1,2] The current understanding of pathophysiology of brain stroke has been mainly derived from the studies involving cell lines and animal models mimicking human stroke. The importance of these models lies in preclinical testing of drugs designed for neuroprotection that may improve functional recovery from stroke. Disappointingly, over a thousand neuroprotective drugs shown effective in animals were found to be virtually ineffective in the treatment of human stroke and clinical trials of many compounds ended prematurely due to disruption of normal brain function and adverse effects except reperfusion with recombinant tissue plasminogen activator (rt-PA). It is approved by FDA for acute ischemic stroke (AIS) treatment with few limitations such as narrow therapeutic time window of 3 h and risk of hemorrhage. In order to examine reasons for failures, it is necessary to develop clear understanding of the pathophysiologic mechanisms involved in

cerebral ischemic damage both in animals and humans. Furthermore, attention has to be focused on reparative processes participating in the surviving tissue.[3]

2. FAILURE IN DEVELOPING NEW DRUGS FOR CEREBRAL ISCHEMIA TREATMENT & THE CHALLENGES FACED

2.1 POTENTIAL PROBLEMS WITH PRIOR ANIMAL & HUMAN STUDIES FOR ACUTE STROKE THERAPIES

Volumes have been written since the 1950s concerning potential neuroprotective agents using animal models. Most of these preclinical studies have demonstrated neuroprotective effects of many pharmacological agents in a variety of ischemic models and in different species. However, when these novel agents have been taken to clinical trials, they have been resoundingly unsuccessful. Some of the major completed and ongoing clinical trials in acute ischemic stroke have been mentioned in Table 1. The reasons for this failure are many and complex, and they may involve timing of the drug administration, window of opportunity, length of time of ischemia, dose of drug given, species, gender differences, age, and underlying diseases. Another issue involves clinical trial design. In spite of potentially effective treatments in animal models, many stroke trials have failed due to naive clinical trial design (i.e., wrong selection criteria for heterogeneous stroke patients, wrong outcome measures, or wrong time window and dose administration of the drug). One must also consider that the animal models utilized for preclinical studies do not reflect the disease as it occurs in humans. This limitation may account for the multiple different models of cerebral ischemia in use today. It arise questions whether the models that have been developed are the best to study research aspects involving mechanisms of injury of disease, and neuroprotection; or are they not reflective of mechanisms of injury and neuroprotection as they pertain to the human disease situation?[3,4,5]

Since the early days of neuroprotective agents in treatment of acute stroke, more than 1000 drugs have been studied and more than 6000 papers describing their neuroprotective efficacy have been published, and yet none of them has been accepted by regulatory authorities to be used for treatment of acute stroke in the United States or the European Union. Positron emission tomography studies has revealed that without early reperfusion, either spontaneously or induced by thrombolysis, the size of the brain infarction can only marginally be reduced with neuroprotective agents because the critically hypoperfused area accounts for the largest proportion (mean 70%) of the final infarct volume.

Table 1: Major completed & ongoing trials in acute ischemic stroke

Mechanism	Compound	Clinical trial	Result
Glutamate receptor antagonists			
NMDA antagonists	Selfotel	ASSIST (abandoned)	No benefit, adverse effects
	Cerestat (Apitagnel)	Apitagnel acute stroke trial	Halted phase III trial; no benefit
	Magnesium	FAST-MAG	Two pilot studies completed; no benefit
	Dextrorphan	Phase II	No benefit
AMPA antagonists	YM872	2 Phase II	Ongoing
	ZK200775	Phase II (abandoned)	Halted due to intolerable sedative effects
Glycine site antagonists	Gavestinel	GAIN international; 2 Phase III	No benefit
	Licostinel	Phase II	Safe with low dose
Free radical scavengers	NXY-059	SAINT I,II	Positive phase III trial; may be effective
	Tirilazad	RANTTAS	No benefit
	Ebselen	Multicentre RCT	Ongoing; better outcome at 1 month, but not at 3 months
Calcium antagonists	Nimodipine	VENUS, TRUST	No benefit
Calcium chelator	DP-b99	Phase II	Ongoing
GABA agonists	Clomethiazole	Clomethiazole acute stroke study	No benefit
	Diazepam	EGASIS	Phase III trial; no benefit
Opioid antagonists	Nalmefene (Cervene)	Cervene stroke study	No benefit
	Naloxone	Phase II	Initial results effective
Anti-inflammatory drugs	Anti-ICAM-1 antibody (Enlimonab)	EAST (abandoned)	Adverse effects
	HU23F2G	HALT	Phase III trial terminated due to negative results
	IL-1 receptor antagonist	Phase II	Initial results effective
Phosphatidylcholine precursor (membrane stabilizer)	Citicoline	Five trials	Improvement observed
NO-pathway modulator	Lubeluzole	LUB-INT-9, LUB-INT-5, LUB-INT-13	No benefit
Acts at the cell membrane and activates cAMP levels	Piracetam	PASS	No benefit on primary endpoint
Growth factor	Erythropoietin	Phase II	Improvement in pilot study
Monoganglioside	GM-1	EST	No benefit

Accordingly, even if neuroprotectants prevent the maturation of the ischemic penumbra to an infarct by half, as in animal models, it would only reduce the final infarct size by 15-20%. It would ask for a trial with thousands of stroke patients to prove such a hypothesis. Animal models have helped us better understand the pathophysiology of ischemic brain damage, but they have otherwise not contributed much to clinical practice so far. One should not expect either that more developed animal models could contribute to emergency stroke care so that a neuroprotective agent can reduce the volume of an infarct in patients with stroke by 50% as they do in rats, at least if the therapy is not combined with thrombolysis or other neuroprotective strategies. If, however, the animal model studies are aimed at

enhancing neuronal regeneration after acute stroke, the landscape changes to a truly bright one. In an innovative animal model, researchers presented strong data for cAMP response element-binding protein (CREB) family transcription factors in recovery from experimental hypoxic ischemic brain damage and that drug can be used to enhance neuronal recovery.[6,7,8] The potential use of animal models for helping to develop AIS therapies should be viewed from several perspectives. It is widely accepted that the pathophysiology of tissue injury in AIS is both simple and complex. Simple because the intraluminal blood flow compromise induced by a thrombus or embolus initiates a complex array of potential contributory mechanisms of cellular and subcellular injury that vary depending on the level of blood flow blockade, the metabolic milieu, genetic environment, and other confounders. The idea of the ischemic penumbra, as suggested by animal studies, is central to the therapeutic time window concept that is being exploited to develop AIS therapies can be potentially effective at later time points, as exemplified by the Desmoteplase preliminary trial. It is only with the availability of increasing knowledge about AIS pathophysiology and temporal evolution provided by animal models those novel therapies at delayed time points can be developed. Animal model-based experiments must be performed to answer specific, goal-oriented questions. Choosing the most appropriate experimental conditions to understand about a drug's therapeutic time window, dose–response relationship, and side effect profile should provide valuable information to the design of subsequent clinical trials. If a drug has a short time window in a model with a well-characterized time period of penumbral survival and a narrow therapeutic index of efficacy, then it is unlikely that the agent represents a good candidate for clinical development. Animal studies should be used to predict likely futility to eliminate drugs not expected to succeed in clinical trials, as well as to identify favorable drugs that should proceed to clinical development. Suggestions that are now extensively used by the pharmaceutical industry for a preclinical assessment paradigm for novel AIS therapies were made by the Stroke Therapy Academic Industry Roundtable (STAIR) group in 1999 and recently expanded on. Conversely, a favorable therapeutic report in stroke models does not guarantee success in clinical development, especially if the clinical trial program has flawed approaches used to assess many drugs in the past. As AIS therapy development evolves toward combination approaches, the performance of good quality preclinical studies will assume importance to help determine optimal dosing regimens for maximal efficacy and to evaluate the potential for drug-drug interactions. It is entirely apt that the combination of improved preclinical assessment and clinical trial design/implementation will conjointly accelerate the development of novel AIS therapies.[9,10] Thus the following may be the potential problems with prior animal studies:

a. Studies used healthy, young animals without comorbid conditions.

b. Animal experiments were performed under anesthesia and involved a surgical method to induce arterial occlusion.

c. The occlusion did not involve a clot.

d. Physiological parameters were not well-controlled.

e. Studies were not done in a randomized, double-blind fashion.

f. Prolonged survival studies were not done to document a persistent treatment effect.

g. Histopathology was the primary outcome and treatment effects on complicated functional outcome measures were not performed.

h. Drug was administered before induction of ischemia or very early after that at a time point not relevant to the clinical condition.

i. Adverse effects of novel neuroprotective agents may have been ignored.[4,11]

2.2 CURRENT STATUS OF NEUROPROTECTIVE DRUGS: THE BASIS FOR STAIR RECOMMENDATIONS

Neuroprotection for ischemic brain injury has emerged recently as a topic of serious biomedical inquiry. A MEDLINE survey (Fig. 1) had revealed that virtually no publications on this topic until the 1990s but a remarkable surge in publications over the past 20 years. In the last few years alone, over 1200 experimental papers and over 500 clinical articles have appeared on this subject. Among clinical trials for ischemic stroke (Fig. 2), those involving thrombolytic, anti-thrombotic, and anti-platelet therapies are more numerous than clinical trials of neuroprotectants. The ability of neuroprotection to flourish as a fruitful field of research depended upon the emergence of a corpus of experimental investigations, beginning in the 1970s, that defined and characterized the pathophysiology of ischemic brain injury and, by implication, pointed the way to potential interventional strategies for thwarting these injurious factors. Reproducible, physiologically controlled animal models of ischemia as well as *in vitro* systems were developed and validated. The cytopathology of ischemic injury was characterized; biochemical and molecular events were elucidated, intracellular mediators identified, and important modulatory influences explored. Taken together, these advances in the understanding provided the fertile milieu in which ischemic neuroprotection could be rationally approached.[11,12] A widespread view of neuroprotection research is that ''everything works in animals but nothing works in people''. Following the message of unsuccessful outcomes of clinical trials in neuroprotection, it is common for nihilistic generalizations to appear that assert ischemic neuroprotection may not be an attainable clinical goal. It is useful, hence, to call attention to numerous studies in experimental

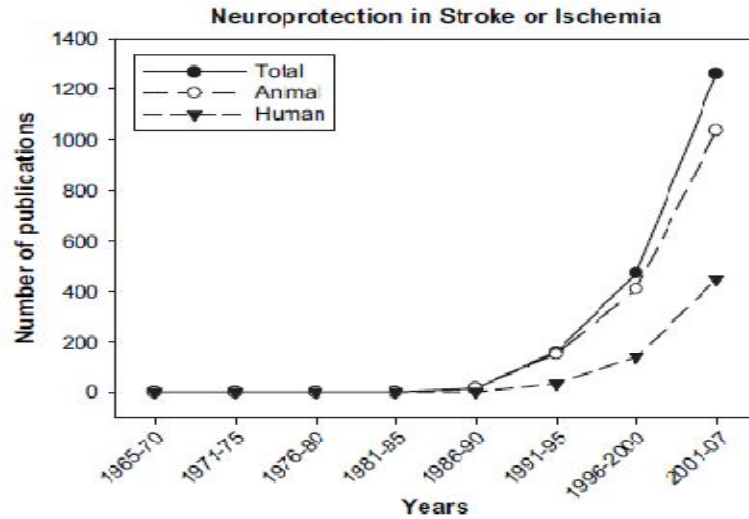animals that provide proof-of-principle that high-grade protection of the ischemic brain is indeed achievable.

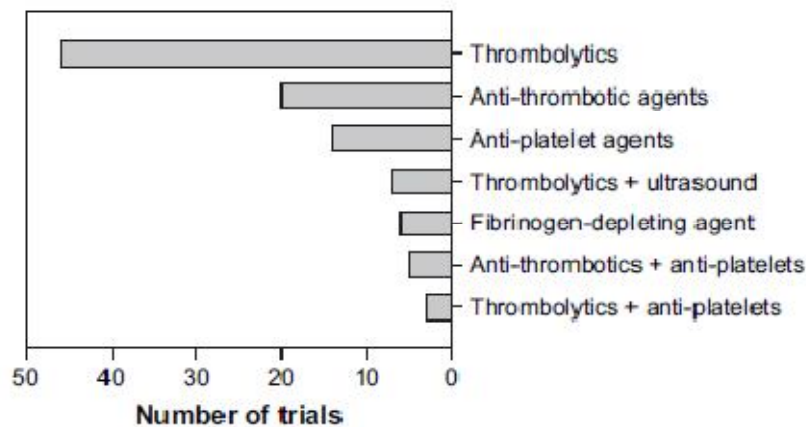**Figure 1: Medline-indexed publications for neuroprotection from 1965-2007**

Figure 2: Clinical trials of key agents in ischemic stroke

that provide proof-of-principle that high-grade protection of the ischemic brain is indeed achievable. It is apparent from the reviews that the preclinical evaluation of many agents that were subsequently brought to clinical trial showed only modest or inconsistent tissue protection. Among 65 agents (other than thrombolytics and anti-thrombotics) that they considered, the mean (±SD) overall extent of tissue protection for the group was a modest 30±19%. In addition, there was variability in the quality of these preclinical studies as reflected in their adherence to the STAIR guidelines. Thus, many agents were brought to clinical trial without a sufficiently relevant evidence-based preclinical foundation. A number of variables come into play in the design and execution of experimental cerebral ischemia investigations and may influence the quality, consistency, and outcome of these preclinical studies.[10,13]

In 2000, the standard form of drug development was to determine its biologic mechanism, efficacy, dosage, and time window in preclinical animal models. The only licensed acute pharmacologic intervention for stroke is rt-PA, which traveled this usual route before proof of its efficacy in clinical trials. Many stroke clinicians have been baffled by the failure of compounds trialed over the past 2 decades, despite strong evidence for efficacy in animal models. Researchers argue that there are many reasons why translation of neuroprotectants from animal models to clinical practice has not occurred and, certainly, this has been the theme of a series of STAIR (Stroke Therapy Academic Industry Roundtable) recommendations. A number of neuroprotectants have had inadequate preclinical testing in differing models, species, and appropriate time windows. For example, there is little justification for human studies of an agent that reduces infarct volumes in a single rodent model by 30% with short time windows. As argued by researchers, there is often a poor understanding of the model itself; knowledge of the presence and duration of the ischemic penumbra is critical. Trial methodology has now become more sophisticated, and negative results are likely to be the result of biologically weak compounds. Also, treatment effect sizes are likely to have been overestimated, and we would not expect an absolute risk reduction of more than 5% for neuroprotectants, substantially lower than for thrombolytic therapy. Researchers have a firm view that larger sample sizes are required for these trials than are presently used. One fact highlighted is that the rigidity of case selection and patient management in clinical trials has driven the standards of acute stroke care. This may be a factor in the lower-than-expected mortality rates in the trials. One exception to the usual pathway of drug development has been the positive results using recombinant factor VIIa to attenuate hematoma growth in patients with primary intracerebral hemorrhage. The biologic plausibility of this approach was based on clinical studies of the dynamics of hematoma growth documented by repeated CT scans rather than animal models. The compound was already in clinical use as a haemostatic agent for another indication. This illustrates the view that although the majority of candidate stroke compounds need to be evaluated in preclinical animal models, there is always a place for compounds already in use for another clinical indication. Despite the history of failure of translation of neuroprotectants into clinical practice, promising trial results have been released for a free radical trapping agent. The development of this agent was based on a rigorous preclinical program, including multiple animal models and careful adherence to the STAIR criteria.[9,14]

2.3 SOLID PRECLINICAL EVIDENCE OF NEUROPROTECTIVE EFFICACY

The minimal preclinical preconditions to be satisfied before bringing a compound to human trial should be:

(a) demonstration of strong, clinically relevant protective efficacy (e.g., 50% or greater infarct-size reduction plus neurobehavioral improvement with a window-to-treatment of at least 3-4 h after onset of ischemia);

(b) proper experimental design of these studies (including monitoring and control of physiological variables, so as to avoid for example, the perplexing influence of brain hypothermia; randomized allocation to treatment groups; blinded outcome assessment; demonstration of long-term protective effect with survival times of at least several days to weeks; proper statistics; etc.); and

(c) replication of positive findings by other laboratories.

Most of the large completed clinical trials were launched without fully satisfying these preclinical milestones.[15]

3. THE STAIR RECOMMENDATIONS

3.1 THE STAIR CRITERIA AND THE QUALITY ISSUE IN NEUROPROTECTION VALIDATING SYSTEM

The translational failure of neuroprotectants for AIS treatment has led to an investigation into the validating system for the efficacy of neuroprotective candidates. Many factors have been discussed as possible reasons for why experimental evidence of efficacy has not translated into efficacy in clinical trials. Some of the frequently raised possibilities are species differences, inappropriate time windows, ineffective drug levels, inability of drugs to cross the blood-brain-barrier (BBB), use of young animals without co-morbidity, failure to model white matter damage, and heterogeneity of stroke subtypes in patients (STAIR, 1999). To date, there have been 5 versions of STAIR criteria (STAIR, 1999; STAIR, 2001; Fisher, 2003; Fisher, 2005; Fisher et al., 2007). These STAIR standards, if followed strictly among researchers, likely could minimize false positive conclusion from preclinical stroke studies.[16,17,18,19]

Although the STAIR standards have been published, the design quality issue in experimental stroke research is still a concern. According to an analysis of published data, many preclinical studies were carried out with lower than average quality. A re-examine of the preclinical data for the failed Stroke – Acute Ischemic NXY Treatment II (SAINT II) trial revealed serious quality issues.[20] The STAIR recommendations, if followed, could have tackled the problems associated with the validating system. The effective implementation of STAIR standards may reduce the inflated efficacy of tested neuroprotectants, i.e., the false positive results; it may not improve the chance of a true positive

discovery of a useful neuroprotectant for treating ischemic stroke. Interestingly the FDA-approved effective recanalisation treatments, including rt-PA treatment, MERCI device for intra-arterial clot removal and Penumbra Aspiration Device, have passed through the same validating system whilst the neuroprotectives failed in their clinical trials. Most likely, the major problem of stroke treatment translation may lie in the early stage of research in identifying therapeutic targets, rather than in the late stage of validating therapeutics. Successful recanalisation therapies must also have considerable effect on some of these factors, so that they can improve the outcome of stroke patients.[11,21]

The almost 2 decades of repeated failures on neuroprotection for AIS has disappointed both academic and industrial fields. Pharmaceutical companies lost huge investments and currently consider neuroprotection for ischemic stroke as a remote goal. A pessimistic feeling also exists in the academic field. Some researchers even challenge the validity of using animal models for stroke research. However, the existing problems in preclinical studies indicate a burning need to improve the quality of experimental stroke research, rather than a challenge against its value to human health and disease treatment. An article reported that neuroprotection without reperfusion may not be possible until there are innovative concepts in protecting ischemic neuronal injury. This again demonstrated the urgent need for a reconsideration of strategies for neuroprotection for AIS treatment.[14,15]

3.2 THE STAIR PRECLINICAL RECOMMENDATIONS

3.2.1 PROBLEMS IN PRECLINICAL STUDIES

STAIR VI met in the aftermath of numerous failed stroke trials in which preclinical data partially after the initial STAIR preclinical recommendations and initial clinical trial results appeared promising. Although there are plentiful potential reasons for disappointing outcomes, an issue that STAIR VI addressed is whether applying externally derived standards to stroke research would improve the likelihood of identifying successful stroke therapies.[20]

In 2006, O'Collins et al. performed an organized review that extracted data for 1025 neuroprotective strategies tested in around 8500 experiments relevant to stroke and published in 3500 articles between 1960 and 2003. This study used a checklist derived from STAIR I to provide an overview of the quality of data available for individual therapies. Testing of only 5 of the 550 drugs reported to be effective in animal models of focal cerebral ischemia fully met this interpretation of the STAIR criteria. One observation in the O'Collins review was a relationship between increasing study quality score (based on adherence to STAIR I criteria) and declining efficacy. It appeared that poor quality

studies overrated efficacy, a phenomenon attributable to bias from lack of randomization and blinding. Systematic review and analysis of the data for 13 alleged neuroprotectants revealed that the presence or absence of randomization to a treatment group, blinding of drug assignment during stroke induction, and blinding of outcome assessments were among the most influential determinants of result. For example, studies of NXY-059 reported that efficacy was appreciably lower in randomized studies (20% vs 53%) and in those that reported allocation concealment between cerebral ischemia induction and outcome assessment (25% vs 54%). In studies of hypothermia, these effects were less striking (36% vs 46% and 38% vs 46%, respectively) but still present. Perhaps due to the frustrations engendered by the failure of translation of apparently efficacious animal neuroprotectants into human stroke therapies and STAIR recommendations, stroke researchers are performing studies of better quality than in the past. However, stroke researchers report random allocation to treatment group in only 35%, allocation concealment in 10%, and blinded assessment of outcome in only 30% of stroke studies. Sample size calculation illustrates the influence of the above issues on experimental results. The probability of detecting a difference between groups is related to the extent of the difference, the variability in the outcome measures, and the number of animals per group. In methodical reviews of the preclinical stroke literature, only 3% of studies report using a sample size calculation. In a worst case scenario if we make the assumptions that the majority of authors really performed but did not report power calculations, used the minimum necessary calculated sample size but did not deem failure to randomly allocate to treatment group as a potential source of falsely large estimates of effect size bias, then 50% of studies might have been underpowered to detect real differences between treatment and control groups. With lack of allocation concealment, the potential for underestimating sample size increases to almost 90% of the studies performed. If the required sample size for detection of a particular effect size in reality is 24 but only 22 animals are used, then potentially all 22 might have been wasted. However, if 26 are used, then the extra 2 have still contributed to useful data.[3,10,21]

Although, such scenarios mostly do not apply to the papers evaluated, without appropriate reporting of sample size calculation, it is not known in which situations it does apply. There is preference for standards in research being well accepted and applied. Clinical trialists adhere to the Consolidated Standards of Reporting Trials (CONSORT) statement, which led to ample improvements in the reporting and conduct of clinical trials as a requirement for publication. On the basis of the available evidence, it would be apt that preclinical testing for the purpose of determining therapeutic efficacy in animal models of stroke should adopt similar standards for conducting and reporting experiments to ensure high-quality unbiased data.[16]

3.2.2 INITIAL STAIR PRECLINICAL RECOMMENDATIONS

a. Satisfactory dose-response curve.

b. Define the time window of treatment in a well-characterized model.

c. Blinded, physiologically controlled reproducible studies.

d. Histological and neurobehavioral outcomes assessed acutely and long-term.

e. Initial rodent studies, then consider gyrencephalic species.

f. Permanent occlusion then transient in the majority of cases.[19]

3.3 UPDATED STAIR RECOMMENDATIONS

The initial STAIR recommendations were used as a benchmark to assess the quality and adequacy of preclinical studies of drugs before clinical trial evaluation. The recommendations likely influenced acute stroke drug development. For example, there are lesser pretreatment studies in the ischemic stroke animal literature compared to the animal studies completed before 1999. Retrospective reviews, however, find that most animal experiments of neuroprotective agents that progressed to clinical trials did not entirely meet the previous recommendations. This suggests that the initial recommendations are not uniformly accepted as an appropriate way to test novel therapeutic candidates. The previous STAIR preclinical recommendations are timely updated, followed by suggested additions.[18,19]

3.3.1 DOSE RESPONSE

The minimum effective and maximum tolerated dose ought to be defined. As stated in STAIR I, there should be a target concentration, a tissue level of effect recognized from animal histology, with behavioral studies giving indication that when the drug is administered to humans there is a realistic prospect of achieving clinical benefit. It should also be acknowledged that the drug in these ranges accesses the target organ.[19]

3.3.2 THERAPEUTIC WINDOW

There is debate about the importance of a therapeutic time window in animals to acute clinical stroke. Some studies suggest that the time window for thrombolysis to rescue ischemic brain tissue may be similar in animals such as rodents and rabbits and humans, although it is model-dependent. Therefore, rodent studies appear relevant in order to address a therapeutic window for thrombolytic and neuroprotective drugs. It should be noted that penumbral imaging using perfusion/diffusion MRI mismatch can be useful to guide the identification of the therapeutic window in a particular model.[19]

3.3.3 OUTCOME MEASURES

Multiple endpoints are important and both histological and neurobehavioral outcomes should be assessed. Histological and behavioral studies must include studies conducted at least 2 weeks or longer after stroke onset to demonstrate a persistent benefit with emphasis on behavioral outcomes in delayed survival studies.[19]

3.3.4 PHYSIOLOGICAL MONITORING

Focal ischemic stroke in animals is usually induced by occlusion of the middle cerebral artery. However, the models of MCAO including the suture and embolic methods are flawed in causing a sustained reduction in blood flow. It is probable in some situations that occlusion may occur but spontaneous reperfusion may ensue, leading to infarct size variability. Basic physiological parameters like blood pressure, temperature, blood gases, and blood glucose must be routinely monitored. Body temperature should be maintained within the normal physiological range. It is vital to monitor cerebral blood flow using Doppler flow or perfusion imaging to document satisfactory sustained occlusion and to monitor reperfusion in transient ischemia models.[19]

3.3.5 MULTIPLE SPECIES

It is recommended that treatment efficacy must be established in at least 2 species using both histological and behavioral outcome measurements. Rodents or rabbits are suitable for initial testing and gyrencephalic primates or cats are desirable.[19]

3.3.6 REPRODUCIBILITY

The positive results obtained in one laboratory need to be replicated in at least 1 independent laboratory before advancing to clinical trials.[19]

Based on subsequent accumulated experience, several added areas are now proposed as follows:

a. The basics of good scientific inquiry should be satisfied by implementing randomization and eliminating outcome assessment bias, defining inclusion/exclusion criteria, and reporting the reasons for excluding animals from the final data analysis, performing apt power and sample size calculations, and disclosure of relevant conflicts of interest.

b. After initial studies reveal positive effects in young healthy animals, further studies in aged animals and those with comorbidities such as hypertension, diabetes, and hypercholesterolemia should be performed if that is the intended population for clinical trials.

c. Efficacy studies should be performed in both male and female animals.

d. Interaction studies with medications regularly used in stroke patients should be performed for advanced preclinical drug development candidates.

e. Appropriate biomarker endpoints such as diffusion/perfusion MRI and serum markers of tissue injury should be included that can be also obtained in clinical trials to indicate that the therapeutic target has been modified.[19]

4. FUTURE PROSPECTS

Cerebral ischemia leads to neurological dysfunction and activate complex signaling cascades, which may depreciate survival mechanisms as a result of cellular homeostatic breakdown. The critical issue regarding the translation of preclinical developments to the successful stroke therapy remains still unresolved. More research efforts and support should be diverted to the field of ischemic energetics so that a break-through in this area could occur in the near future. Despite the complexities of cellular events in stroke damage, researchers have suggested several targets starting from disruption of NMDA receptor interaction, hence excitotoxicity, combating oxidative stress through SOD, etc., modulation of cell signaling (CaMK/MAPK pathways), inhibition of inflammatory reactions and cellular damage mechanisms (caspases, HSP70, Bcl-2 family proteins, etc.) and application of gene therapy, which may hold promise in the near future to revive the brain function and ultimately survival.[21] Nevertheless, continuous cellular and molecular research in this crucial area is indispensable, to generate important information for underlying mechanisms of neuronal survival/damage and for the development of suitable and effective neuroprotective strategies. It is possible that manipulating intracellular energy state with novel approaches, pharmacological preconditioning, and controlled torpid state may facilitate the discovery of an effective treatment for ischemic strokes.

Although the initial STAIR recommendations were useful in improving many features of preclinical testing, they have not yet been shown to predict whether any drug will improve outcome in pivotal efficacy phase III trials. Meanwhile, the updated and amended STAIR preclinical recommendations provide a basis for further thinking, careful discussions, and inter-laboratory collaborations regarding how to best enhance the usefulness of preclinical studies of alleged acute stroke therapies. However, it must be accepted that fulfilling them does not guarantee success in clinical trials. Rigorous and complete preclinical testing should provide reassurance that there is potentially a greater chance for success in clinical trials, assuming that the clinical development program is also conducted according to currently accepted standards. In addition to these issues, success will certainly hinge on the

education of the public and health professionals to respond swiftly to the onset of symptoms of cerebral ischemia so that early treatment can be initiated.

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