1,026 Experimental Treatments in Acute Stroke

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Objective: **Preclinical evaluation of neuroprotectants fostered high expectations of clinical efficacy. When not matched, the question arises whether experiments are poor indicators of clinical outcome or whether the best drugs were not taken forward to clinical trial. Therefore, we endeavored to contrast experimental efficacy and scope of testing of drugs used clinically and those tested only experimentally.** ***Methods:*** **We identified neuroprotectants and reports of experimental efficacy via a systematic search. Controlled in vivo and in vitro experiments using functional or histological end points were selected for analysis. Relationships between outcome, drug mechanism, scope of testing, and clinical trial status were assessed statistically.** ***Results:*** **There was no evidence that drugs used clinically (114 drugs) were more effective experimentally than those tested only in animal models (912 drugs), for example, improvement in focal models averaged 31.3 ± 16.7% versus 24.4 ± 32.9%, $p > 0.05$, respectively. Scope of testing using Stroke Therapy Academic Industry Roundtable (STAIR) criteria was highly variable, and no relationship was found between mechanism and efficacy.** ***Interpretation:*** **The results question whether the most efficacious drugs are being selected for stroke clinical trials. This may partially explain the slow progress in developing treatments. Greater rigor in the conduct, reporting, and analysis of animal data will improve the transition of scientific advances from bench to bedside.**

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A common perception of neuroprotection research is that everything works in animals but nothing works in people. This perception has been reinforced again and again by reports of unsuccessful or mixed outcomes in trials of candidate neuroprotectants in acute stroke patients. If animal experiments are indeed unable to inform clinical decision making, then serious doubts are raised about the utility of animal models of stroke and about the ethics of continuing current animal experimentation practices.[1]

In response to this challenge, several excellent reviews and commentaries have tackled the issue of the apparent failure to translate neuroprotection successes from the laboratory to the clinical setting (for examples, see previously published studies[2–7]). Such commentaries have questioned the appropriateness of the experimental animals (eg, age, sex, comorbidities, comparative anatomy, sample sizes), the stroke model (eg, anesthesia, hypothermia, glucose, reperfusion), the outcome measures (histology vs functional deficits and death), study quality (blinding, randomization), and clinical patient selection and dosing (patient heterogeneity, inappropriate dose, and time window). These reviews have provided a solid framework for sharpening experimental and clinical trial design, but, by and large, they have been qualitative rather than quantitative in nature. Additionally, only recently has the impact of study design and quality been examined in relation to efficacy in experimental models of stroke.[8–10]

We set out to examine whether it is indeed true that everything produces neuroprotection in animals, and to compare the experimental efficacy of those interventions taken forward to clinical use with that of drugs tested only in the laboratory. A broad definition of neuroprotection was adopted, encompassing any agent or treatment tested in an animal model of stroke, whether administered with the intent of preventing neuronal death, restoring blood flow, or merely investigating the mechanisms of damage in stroke. Thus, of the many ways one can define neuroprotection (see Table 1), primacy was given to the evaluation of the final effect of a drug on the preservation of brain function, irrespective of intended cellular target, purpose of administration, or clinical classification of the drug.

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Table 1. Definition of a Neuroprotectant

Definition	Description
Clinical	Agent administered after acute stroke to preserve neurons, in contrast with agents that restore blood flow (thrombolytics). *Limitation*: Absence of strong logical argument as to why thrombolytics should be considered a discrete class, whereas the neuroprotectives class should include a myriad of agents themselves with other clinical uses (eg, antidepressants, fluid replacement). Furthermore, whereas the immediate target of thrombolytics might be blood flow, the ultimate effect of interest is normalization of brain function and behavior. The direct effect of thrombolytics on neural tissue is also attracting greater attention.
Properties of drug	Agent containing chemical or physical properties known to be associated with neuroprotective effects. *Limitation*: Such properties are generally yet to be elucidated.
Cellular target	Agent targeting cellular pathways that control neuronal fate to preserve brain function. *Limitation:* Greater understanding required regarding which cells must survive to preserve brain function (eg, neurons, oligodendroglia, microglia) and of how the fate of individual cells relates to the overall survival of brain tissue. More information is also needed regarding how the preservation of processes governing the recruitment and disengagement of brain networks relates to the behavior thought to be subserved by these networks.
Purpose of administration	Agent administered with the purpose of preserving brain function. *Limitation:* Drugs administered for other purposes, eg, fluid replacement, antidepressants, thrombolytic, may also affect the preservation of brain function.
Final effect	Agent whose final effect is to preserve brain structure or function. *Limitation*: Nonmechanistic, empirical approach does not facilitate further drug development.

A further aim was to examine the scope of preclinical testing to which drugs have been subjected, with a view to assessing the adequacy of our procedures for selecting drugs to carry forward to clinical trial. Here, scope of testing refers to the range of preclinical experimental procedures to which an intervention has been subjected (eg, range of species and stroke models in which the drug was tested). Scope of testing, together with quality control within individual experiments (eg, via blinding and randomization) form the twin pillars of the Stroke Therapy Academic and Industry Roundtable recommendations.[11] These recommendations relate to the benchmarks for preclinical evidence that should be met before an experimental treatment is considered for clinical trial and represent an important consensus on preclinical standards reached between several distinguished researchers in the field of stroke.

Neuronal injury in stroke is thought to result from a surge in activation of interlinking pathophysiological pathways, with different pathways possibly predominating in the core and at the cusp of ischemic damage.[12–14] Neuroprotective treatments in stroke typically target one or more of these mechanisms of damage; hence, we have also investigated whether efficacy in experimental stroke was substantially different in any of the major categories of therapeutic agents.

The main aims of the review were therefore (1) to identify agents tested in animal neuroprotection models and those treatments given to acute stroke patients; (2) to compare the overall quality of evidence and experimental efficacy of those treatments that have been given to acute stroke patients (clinical treatments) and those agent or strategies that have not progressed beyond the experimental phase (experimental agents); and (3) to compare the experimental neuroprotective efficacy of the main classes of neuroprotective agents. Because neuroprotective agents were being evaluated primarily for their application to stroke, greater emphasis was placed on the identification and analysis of the efficacy demonstrated in models of focal ischemia (rather than global ischemia or cell culture methods) because focal models are thought to more closely mimic human stroke.[15]

Materials and Methods

Identification of Putative Neuroprotective Drugs

Drugs or treatments used in experimental stroke were identified by (1) PubMed search for "neuroprotection"; (2) PubMed search for "cerebral ischemia" from 1960 to 1980 (to capture agents tested prior to the use of the keyword "neuroprotection"), (3) search of the test and reference lists of articles identified by (1) and (2). Drugs used in clinical stroke were identified by searching of clinical trial databases[16,17] and clinical trial reviews[18,19] and from the text and reference lists of articles identified in the methods described above. Because the focus was on whether the intervention has been trialed in humans and not whether the clinical trial was well conducted, reports of any trial in humans were used to demarcate the intervention clinical trial status.

Data Sources

Reports of efficacy in experimental stroke were identified via PubMed and by hand-searching of relevant journals. For each drug, a PubMed search was conducted using the search criteria "(drug name) AND (cerebral ischemia OR stroke OR neuroprotection)." Where no results were found, the search was repeated using only the drug name. Information on the physiological effects of drugs was obtained from the Merck

Table 2. Models of Cerebral Ischemia

Model	Method of Induction	
Focal ischemia	Mechanical	Clip, ligation by external filament, internal filament, inflatable cuff
	Thermal	Electrocauterization
	Embolic	Microsphere, macrosphere, autologous clot, fibrin clot, polyvinyl acetate
	Chemical	Endothelin-1, Rose Bengal photochemical dye, arachidonic acid, adenosine 5′ phosphate and epinephrine, FeCl3
Global ischemia	Respiratory	Potassium cyanide (KCN), asphyxia, carbon dioxide, nitrogen
	Thermal	Electrocausterization
	Mechanical	Clip, tourniquet, balloon compression, decapitation, ligation, intracranial pressure elevation
Culture	Apoptosis	Staurosporine, C2-ceramide, paclitaxel, low potassium, etoposide, tunicamycin, serum deprivation, Bleomycin sulfate, 3-morpholinosydnonimine, SIN-1, sodium nitroprusside (SNP), *S*-nitroso-*N*-acetylpenicillamine (SNAP)
	Calcium	Thapsigargin
	Inflammation	Lipopolysaccharide, chromogranin A
	Ischemia	Anoxia, hypoxia, oxygen, and glucose deprivation, veratridine, sodium cyanide, iodoacetic acid, sodium azide (cytochrome oxidase inhibitor), endothelin-1
	Oxidation	H_2O_2, iron, heme, photochemical stress, glutathione depletion, superoxide, naphthazarin, antimycin A, rotenone, 3-morpholinosydnonimine, sodium nitroprusside (SNP), *S*-nitroso-*N*-acetylpenicillamine (SNAP)

Index, online chemical databases, and experimental and review papers. Foreign language reports were included only when published with an English abstract.

Study Selection

Drug efficacy studies in established in vivo and in vitro models of neuroprotection were considered for inclusion (see Table 2). Both focal and global models of ischemia were included, but experiments performed in nonstroke models of neuroprotection (eg, Parkinson's disease and epilepsy) were excluded. In vitro models of neuroprotection designed to model the pathophysiology of stroke were also included and typically involved the application of a toxic stimulus (eg, glutamate, oxygen and glucose deprivation, hydrogen peroxide) to a neuronal culture or coculture of neurons and astrocytes, to hippocampal or cortical slices or to retinal explants. Controlled experiments measuring outcome using a functional (behavioral), histological, survival, electrophysiological, or imaging end point were retained. For focal ischemia studies, we included only those studies reporting outcome as infarct volume (measured histologically or by brain imaging) or a behavioral score. For global ischemia, we included studies reporting survival; the extent of cell death; neurobehavioral scores; or normalization of electroencephalogram. In keeping with the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations,[11] reports restricted to consideration of mechanism of drug action, gene expression patterns, cerebral water content, blood–brain barrier function, blood flow changes, and restoration of metabolism were excluded because these represent surrogate end points having complex relationships with neuronal survival and neurological impairment.

Data Extraction

For each study, the following information was collected: type of experimental model, drug or intervention, and outcome. A more comprehensive data set was compiled for focal ischemia experiments, with the following information also extracted: animal (species sex, age, comorbidities); stroke model (method of induction); intervention (dose, time treatment, mode of delivery), and outcome (end point, time measured). Information regarding focal ischemia experiments was taken from full publications (where available) but for other experiment types was generally extracted from published abstracts.

Each experiment was given a score of −1, 0, or 1 depending on whether the treatment resulted in an outcome that was significantly worse, the same, or better than that in the control group, respectively. Where the drug had also been tested in focal models of ischemia and the infarct volume (IV) reported, the average level of protection (%) was also reported. Thus, for N studies:

$$\text{Average level of protection (\%)} = \frac{1}{N}\sum_{n=1}^{n=N}\left(1 - \frac{\text{Treatment Group Infarct Volume}}{\text{Control Infarct Volume}}\right) \times 100$$

Scoring System for Scope of Testing

A scoring system was designed to measure the diversity of evidence supporting each drug, based on the STAIR recommendations. Each drug was assigned a score (0–10) to reflect how widely it has been tested in preclinical models of stroke (Table 3). Although the criteria largely reflect the STAIR recommendations, several departures were made; testing in nonhuman primates was not included as a criterion because the superior validity of such models is not well established. Additionally, because the focus of the review was the performance of multiple drugs over multiple experiments, the scoring system did not include measures relating to quality control within individual experiments (eg, randomization and blinding) but instead focused on the scope of testing across experimental models.

Table 3. Quality of Evidence in Experimental Stroke Scale

Item	Item	Description
1.	Laboratory setting	Focal model tested in two or more laboratories
2.	Animal species	Focal model tested in two or more species
3.	Health of animals	Focal model tested in old or diseased animals[a]
4.	Sex of animals	Focal model tested in male and female animals
5.	Reperfusion	Tested in temporary and permanent models of focal ischemia
6.	Time window	Drug administered at least 1 hour after occlusion in focal model
7.	Dose response	Drug administered using at least two doses in focal model
8.	Route of delivery	Tested using a feasible mode of delivery (eg, not intracisternal or intraventricular, cortical transplant or graft only)
9.	Endpoint	Both behavioral and histological outcome measured
10.	Long-term effect	Outcome measured at 4 or more weeks after occlusion in focal models

Not given in order of priority.

[a]Diabetic, hypertensive, aged, hyperglycemic.

Statistics

Excel and Systat 11 were used to analyze the data. All means are presented as mean ± standard deviation (SD). A 10(drug mechanism) × 2(clinical trial status) analysis of variance (ANOVA) was undertaken to evaluate the effect of clinical trial status and mode of drug action on outcome in focal models of ischemia. Scope of testing was analyzed separately because it did not meet the assumptions for ANOVA.

Results

Number of Neuroprotection Experiments and Clinical Trials

A total of 8,516 experimental results were extracted from approximately 3,500 papers (published between 1957 to 2003). Of these, 962 experimental results were excluded because they related to nonstroke neuroprotection models; thus, the analysis is based on 7,554 experimental results from models of focal ischemia (3,867 results), global ischemia (1,546 results), and culture (1,341 results). The 1990s showed a marked growth in reports of neuroprotection experimentation (Fig 1). At the same time, there was a less marked increase in reports of the first clinical application of candidate neuroprotective drugs (Fig 2).

Drug Identification

The initial search identified 1,150 interventions; 124 were excluded from further analysis: 56 because reports of their effects related only to mechanism or used noneligible end points, and 68 because the literature related to neuroprotection in models of disease other than stroke. This analysis therefore is based on data for 1,026 candidate stroke drugs, of which 912 have been tested in animal models only, 97 have been tested in both animal models and clinical trials, and 17 have been tested only in humans. For 51% of the 114 drugs tested in humans, the first report of its clinical use was published before the first report of its use in a focal ischemia model of stroke, and 42% of these drugs were used clinically before the first report of testing in a global or focal model.

Efficacy in Ischemia Models

Of the 1,026 agents, 603 (59%) had been tested in focal ischemia models, 256 (25%) had been tested in both focal and global ischemia models, and 24 (2%) had been tested in focal, global, and cell culture mod-

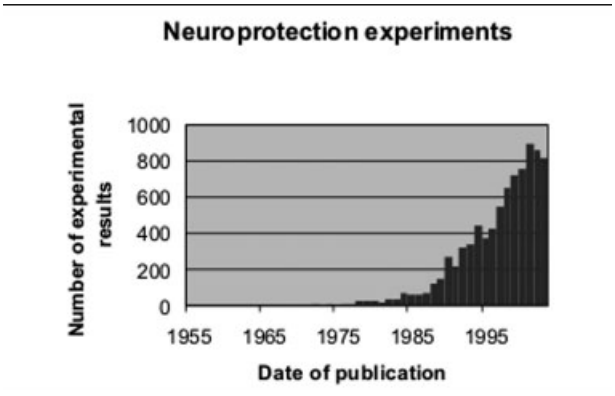

Fig 1. Neuroprotection experiments identified from published reports (1955–2003).

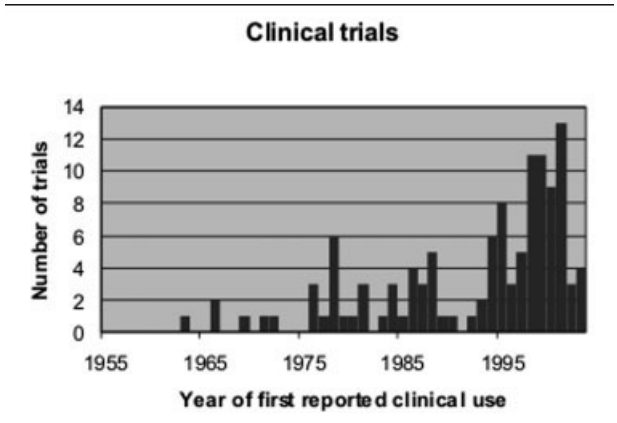

Fig 2. First reported clinical trials of inventions in acute stroke patients (1955–2003).

els. Neuroprotective efficacy was superior to the control condition in 62% of focal ischemia experimental results, and no different or inferior to the control condition in 34% and 4% of cases, respectively. In global ischemia models, neuroprotective efficacy was superior to the control condition in 70% of experimental results, and no different or inferior to the control condition in 26% and 4% of cases, respectively. In culture models, neuroprotective efficacy was superior to the control condition in 74% of experimental results, and no different or inferior to the control condition in 23% and 3% of cases, respectively.

Table 4 shows the analysis of animal experiment data from these comparisons for those drugs tested in clinical trial. For each drug, the number of positive, neutral, and detrimental experimental outcomes are reported for each of the culture, focal, and global models.

Experimental Efficacy in Focal Models of Ischemia

Because ANOVA is very sensitive to extreme results, one study using a hamster model of focal ischemia was excluded because it showed an unusual 1,250% increase in damage using tissue plasminogen activator (t-PA) and 2,500% increase in damage using streptokinase.[20] The average level of neuroprotection in focal ischemia models was not significantly different ($F[1,416] = 2.684$, $p > 0.05$) between purely experimental agents (mean, 24.4%; SD, 32.9%, n = 351) and treatments also used clinically (mean, 31.35%; SD, 16.7%, n = 66). Furthermore, the outcome in focal ischemia experiments was not related to the primary hypothesized mechanism of drug action (Fig 3; $F[9,416] = 1.210$, $p > 0.05$).

Scope of Testing and Its Relationship to Experimental Efficacy

Scope of testing and its relationship to experimental efficacy was examined separately because it did not satisfy the assumptions for analysis of variance. Figure 4 shows the average level of neuroprotection (maximum 100%) versus the number of STAIR criteria satisfied (maximum 10 points) for each drug. As the quality of evidence increased with more STAIR standards being met, the average level of neuroprotection of drugs tended to regress toward the overall mean of approximately 25%. Interventions tested in a broad range of experimental paradigms and demonstrating a superior level of efficacy included NXY-059 and hypothermia (see Fig 4).

Discussion

In the search for an effective treatment for stroke and other neurological conditions, researchers have drawn from sources as diverse as Malaysian pit viper venom,[21] Polynesian ceremonial beverages,[22] aged garlic,[23] and sea snail peptides.[24] More than 1,000 such drugs and nonpharmacological strategies have been tested for their neuroprotective efficacy in animal models, and at least 114 have been administered to acute stroke patients. Efficacy in focal ischemia ranged from 94% protection to a twofold increase in damage in the intervention versus the control condition.

Those drugs given to acute stroke patients were not distinguished by superior efficacy in animal models of focal ischemia compared with all drugs that have been tested only experimentally. Given that the purely experimental class of agents included drugs used to induce comorbid disease states in animals (eg, diabetes, inflammation), this raises the issue of whether we are in fact selecting the best drugs to carry forward to clinical trial.

The unremarkable experimental track records of some drugs given to patients may be explained by several factors. Historically, less emphasis may have been placed on animal experimentation, and where it was undertaken, the outcomes used may have related to metabolism or blood flow rather than neuroprotection. Second, the conditions in clinical trials often fail to replicate the conditions under which drugs have been found to work in animals, especially with regard to the time of administration of treatments. Attempts to design clinical trials having greater regard to the performance of drugs in animal models may assist in the translation of results. Third, more extensive animal experimentation may have been undertaken only after clinical use in an attempt to explain negative clinical results. Finally, this review encompassed all drugs given to patients in the context of acute stroke with the purpose of improving brain function: some of these drugs may not have been considered "neuroprotectants" in the narrow sense, and for this reason they may not have been tested in animal models of ischemia.

Recent developments in the formulation of guidelines for design and interpretation of animal experimentation may assist the selection of candidate drugs for clinical trial. Using a scale based on the STAIR recommendations for preclinical testing, it is clear that experimental efficacy ratings must be viewed in light of the scope of evidence supporting the use of a drug. An intervention should be considered for clinical trial only when there is both a high level of experimental efficacy and a diverse body of evidence supporting its clinical application. In this review, NXY-059 and hypothermia performed well against both these criteria, and early reports from the clinical trial of NXY-059 suggest a potential alleviation of stroke-induced disability.[25] Other considerations relating to cost, drug safety, regulatory approval, intellectual property status, and putative drug mechanism are also clearly relevant, but it may be that such issues have overshadowed neuroprotective efficacy in the past.

Table 4. Preclinical Evidence for Drugs Administered to Acute Stroke Patients

Name	First Trial[a]	Mechanism	Level of Protection (%)[b]	N[c]	Experimental Model[d]									STAIR Quality[e]
					Focal			Global			Culture			
					+	O	−	+	O	−	+	O	−	
Excitotoxicity														
ACEA 1021 (licostinel)	1997	NMDA glycine site antagonist	37	25	19	6	0	0	3	0	2	0	0	7
ARL 15896(AR-A15896AR)	1999	NMDA antagonist	39	15	10	8	0	8	8	0	2	0	0	10
Baclofen	2001	GABA-B agonist	—	0	1	1	0	3	2	0	0	0	0	1
BIII 890 CL	2001	Sodium channel block	27	6	6	0	0	0	0	0	1	0	0	4
BMS-204352	1998	Potassium channel opener	14	9	7	1	0	0	0	0	0	0	0	5
CGS 19755 (selfotel)	1995	NMDA antagonist	47	2	4	1	1	4	1	0	2	1	0	7
Clomethiazole (CMZ, Zendra)	1996	GABA agonist	42	7	8	2	0	3	1	0	0	0	0	9
CNS1102 (Cerestat, aptiganel)	1994	NMDA ion channel blocker	51	11	11	2	0	0	0	0	0	0	0	6
CP101.606-27	1999	NMDA ion channel blocker	61	3	3	0	0	0	0	0	0	0	0	8
Dextrorphan	1994	NMDA ion channel blocker	50	17	13	6	0	6	0	0	3	0	0	7
Eliprodil (SL 82.0715)	1994	NMDA polyamine antagonist Sigma ligand	48	4	6	0	0	3	2	0	1	0	0	6
Diazepam (valium)	2000	Benzodiazepine	—	0	0	1	0	12	1	0	1	0	0	1
Fosphenytoin	1995	Sodium channel blocker, Glutamate release inhibitor.	—	0	0	0	0	1	0	0	0	0	0	0
Gavestinel (GV150526A)	1999	NMDA glycine antagonist	60	18	8	6	0	0	0	1	1	0	0	3
Glycine	1996	NMDA antagonist	—	0	0	0	0	0	1	0	2	0	1	0
Lifarizine (RS-87476)	1995	Sodium/calcium channel blocker	46	8	5	4	0	3	0	0	0	0	0	5
Lubeluzole	1994	Sodium/calcium channel blocker NOS inhibitor	23	19	13	8	0	3	2	0	4	2	0	6
Magnesium sulphate	1993	NMDA ion channel blocker. Calcium antagonist	35	10	11	0	0	17	9	1	1	1	0	7
Nalmefene	1998	Opiod antagonist	—	0	0	0	0	1	0	0	0	0	0	0
Naloxone	1981	Opiod antagonist	29	7	8	7	0	5	7	2	2	0	0	8
Nicergoline	1985	α2 adrenoceptor agonist Enhances glutamate uptake	—	0	1	0	0	8	3	0	0	0	0	2
NPS 1506	1998	NMDA ion channel blocker	—	0	6	2	0	0	0	0	1	0	0	4
NS1209/SPD 502	1999	Gluamate antagonist	44	2	2	0	0	0	0	0	0	1	0	3
Remacemide	1994	NMDA ion channel blocker Sodium channel blocker	49	1	1	0	0	0	0	0	1	0	0	2
S-1746	2001	NMDA glycine/AMPA antagonist	—	0	0	0	0	0	0	0	0	0	0	0
Sipatrigine (BW619C89)	1995	Sodium channel antagonist Glutamate release inhibitor	41	37	40	4	0	1	0	0	1	0	0	8
YM872	1999	AMPA antagonist	27	32	22	8	0	0	0	0	1	0	0	7
YM90K	1997	AMPA antagonist	31	23	19	6	0	5	0	0	0	1	0	8
ZK200775 (MPQX)	1997	AMPA antagonist	19	21	12	9	0	1	0	0	0	1	0	6
Antiinflammatory														
Dexamethasone	1971	Glucocorticoid, antiinflammatory	19	11	7	8	1	5	6	6	1	0	0	8
Enlimomab (anti–ICAM-1 antibody)	1996	Leukocyte migration and adhesion inhibitor	14	9	6	7	1	0	0	0	0	0	0	8
FK506 (pacrolimus)	2004	Immunosuppressant	31	72	52	27	0	20	12	0	12	6	0	9
Fludrocortisone	1999	Mineralocorticoid	—	0	0	0	0	0	0	0	0	0	0	0
Hu23F2G (LeukArrest)	1999	Leukocyte adhesion inhibitor	57	1	1	0	0	0	0	0	0	0	0	1
Indomethacin	2001	Cyclooxygenase inhibitor	23	2	3	2	0	11	10	0	2	2	0	4
LDP-01 (Anti–β-2-integrin antibody)	1999	Leukocyte adhesion and migration inhibitor	—	0	0	0	0	0	0	0	0	0	0	0
Neutrophil inhibitory factor (rNIF, UK-279.276)	2000	Neutrophil inhibitor	31	12	8	4	0	0	0	0	0	0	0	4
Paracetemol (acetaminophen)	1987	Analgesic/antipyretic COX inhibitor	8	1	0	1	0	0	0	0	0	0	0	1
Ganglioside GM1	1984	Metabolism, growth	4	1	6	4	0	9	2	0	0	0	0	8
Insulin	1993	Lowers glucose	16	5	4	1	2	8	1	0	0	0	0	8
Xanthinol nicotinate (Sadamin)	1977	Vitamin B(3): metabolic enhancer	—	0	0	0	0	1	1	0	0	0	0	0
Antioxidant														
Ebselen (Harmokisane)	1998	Free radical scavenger	27	9	10	6	0	0	0	0	2	0	1	7
MCI-186 (Edaravone)	2001	Free radical scavenger	−24	8	7	5	0	4	1	1	0	0	0	7
Nicaraven (N,N′-propylenedinicotinamide)	2001	Free radical scavenger	17	4	2	2	0	0	0	0	0	0	0	3
NXY-059	2001	Free radical scavenger	43	27	24	5	0	0	0	0	0	0	0	10
Tirilazad (U74006F)	1994	Free radical scavenger	26	16	11	8	0	6	5	0	4	2	0	8
Antiapoptotic/regeneration														
Basic fibroblast growth factor (trafermin. Fiblast)	1998	Growth factor	29	35	22	19	0	5	2	0	14	3	0	10
Erythropoietin	2002	Antiapoptosis, oxygen delivery	39	9	11	2	0	12	15	0	11	11	0	9
PS519/MLN519	2000	Proteasome inhibitor	32	14	11	3	0	0	0	0	0	0	0	5

Table 4. Continued

Name	First Trial[a]	Mechanism	Level of Protection (%)[b]	N[c]	Experimental Model[d]									STAIR Quality[e]
					Focal			Global			Culture			
					+	O	−	+	O	−	+	O	−	
Calcium/adrenergic modulators/ antihypertensives														
Atenol (Tenormin)	1988	Beta blocker	—	0	0	0	0	0	0	0	0	0	0	0
Candesartan cilexetil (TCV-116, Blopress, CV-11974)	1999	AT1 receptor antagonist Anti-hypertensive	34	5	4	1	0	0	1	0	0	0	0	3
Cyclandelate	1966	Vasodilator (calcium modulator)	—	0	0	0	0	0	0	0	0	0	0	0
DP-b99 (DP-BAPA)	2000	Calcium chelator	—	0	0	0	0	0	0	0	0	0	0	0
Flunarizine	1990	Calcium channel blocker	−6	3	4	1	1	24	7	1	3	0	0	6
Nicardipine	1988	Calcium antagonist	11	6	8	10	0	12	15	0	1	2	0	7
Nicergoline	1985	Alpha2 adrenoceptor agonist Enhances glutamate uptake	—	0	1	0	0	8	3	0	0	0	0	2
Nimodipine	1984	Calcium channel blocker	26	37	24	28	0	11	10	0	1	2	0	9
Papaverine	1976	Calcium channel blocker	−3	1	0	1	0	0	0	0	0	0	0	2
Propranolol	1988	Beta-adrenergic blockade, Membrane stabilization	34	4	3	8	0	0	0	0	0	0	0	6
PY 108-068	1986	Calcium antagonist	—	0	2	0	0	0	0	0	0	0	0	5
S-0139 (SB-737004)	1999	Endothelin antagonist	36	4	3	1	0	0	0	0	0	0	0	2
Vinpocetine (ethyl apovincaminate)	1986	Calcium inhibitor, Vasodilator, Sodium blocker.	42	1	1	0	0	9	0	0	0	0	0	
Thrombolytic														
Abciximab (reopro, c7E3 Fab)	1998	Antiplatelet: glycoprotein inhibitor	27	2	1	1	0	0	0	0	0	0	0	3
Aminophylline	1976	Phosphodiesterase inhibitor	—	0	0	0	0	0	0	0	1	0	0	0
Anerod	1983	Fibrinogen depleting	21	4	4	1	0	0	0	0	0	0	0	4
Argatroban	1986	Anticoagulant	11	4	3	3	0	1	1	0	0	0	0	5
Aspirin	1995	Antiplatelet	31	19	9	13	0	0	2	0	10	0	0	8
Batroxobin (defibrase, DF-521)	1995	Fibrinogen depleting	—	0	4	0	0	1	0	0	1	0	0	1
Certoparin	2000	Anticoagulant	—	0	0	0	0	0	0	0	0	0	0	0
Dalteparin	2000	Anticoagulant	—	0	0	0	0	0	0	0	0	0	0	0
Defibrotide (polydeoxyribonucleotide)	1989	Antiplatelet: glycoprotein inhibitor	—	0	0	0	0	1	0	0	0	0	0	0
Desmoteplase (DSPA)	2002	Antithrombotic	—	0	0	0	0	0	0	0	0	0	0	0
Enoxaparin	2003	Antithrombotic	25	25	12	13	0	0	0	0	0	0	0	6
Eptifibatide (cromafiban; Integrilin)	2003	Antiplatelet: glycoprotein inhibitor	—	0	0	0	0	0	0	0	0	0	0	0
Heparin	1979	Anticoagulant	32	17	10	10	3	2	0	0	1	0	0	8
Nadroparin	1995	Antithrombotic	—	0	0	0	0	0	0	0	0	0	0	0
Org 10172 (danaparoid, Orgaran)	1997	Antithrombotic	—	0	0	0	0	0	0	0	0	0	0	0
Pentoxifylline	1981	Improve capillary flow	—	0	0	1	0	3	0	0	0	0	0	2
Propentofylline (HWA 285)	1992	Phosphodiesterase inhibitor	37	7	9	2	0	7	2	0	2	1	0	6
Prosatacyclin	1984	Antiplatelet: eicosanoid Vasodilator	−6	1	1	1	0	9	1	0	1	0	0	5
Prourokinase	1998	Antithrombotic	55	12	12	0	0	0	0	0	0	0	0	3
RPR 109891	1998	Antiplatelet glycoprotein inhibitor	—	0	0	0	0	0	0	0	0	0	0	
n-PA/tPA (alteplase)	1988	Antithrombotic	4	86	52	38	11	0	1	0	1	0	0	9
Streptokinase	1963	Thrombolytic	−525	6	1	4	5	0	0	0	0	0	0	6
Tinzaparin	1998	Anticoagulant	—	0	0	0	0	0	0	0	0	0	0	0
Tirofiban (MK-383, aggrastat)	2001	Antiplatelet: glycoprotein inhibitor	—	0	0	0	0	0	0	0	0	0	0	0
TNK (tenecteplase)	2000	Thrombolytic agent	35	2	2	0	0	0	0	0	0	0	0	4
Triflusal (2-acetoxy-4-trifluoromethylbenzonic acid)	2001	Arachidonic acid metabolism inhibitor (antiplatelet)	—	0	1	2	0	0	0	0	0	0	0	2
Urokinase	1976	Thrombolytic	53	12	13	1	0	0	0	0	0	0	0	7
Nootropic/stimulant														
Amphetamines	2003	Stimulant	−3	1	1	2	0	2	1	0	0	0	0	7
Cerebrolysin	2001	Nootropic	—	0	1	1	0	6	1	0	1	0	0	4
Citicoline (CDP choline)	1987	Membrane precursor, antioxidant Vascular insufficiency Immunostimulatory Nootropic	25	13	4	9	0	8	1	0	0	0	0	8
EGB-761 (Gingko biloba extract)	1995	MAO inhibitor Antiplatelet. Antioxidant Reduces leukocyte activation Increases cerebral blood flow	25	15	13	3	0	4	0	0	10	1	0	7
Hydergine	1978	Nootropic, antioxidant.	—	0	0	0	0	2	0	0	0	0	0	0
Nafronyl oxalate (naftidrofuryl)	1978	Serotonin antagonist	38	5	6	2	0	2	0	0	1	0	0	7
Piracetam	1988	AMPA (NA+) modulator	39	5	4	1	0	6	2	0	0	1	0	4
Semax	1997	Derivative of ACTH-4-10	—	0	0	0	0	4	0	0	1	0	0	0

Table 4. Continued

Name	First Trial[a]	Mechanism	Level of Protection (%)[b]	N[c]	Experimental Model[d]									STAIR Quality[e]
					Focal			Global			Culture			
					+	O	−	+	O	−	+	O	−	
Fluid regulation														
Glycerol	1972	Hyperosmolar agent	—	0	0	2	0	1	2	0	0	0	0	0
Dextran	1969	Hemodilution	34	7	4	5	1	0	0	1	0	0	0	7
Hydroxyethyl starch pentastarch	1980	Hemodilution	23	3	4	3	1	0	0	0	0	0	0	8
Mannitol	1978	Hyperosmotic agent. Reduces edema and ICP	34	19	10	15	1	8	5	1	0	0	0	8
Oxygen delivery[f]														
Diaspirin cross-linked hemoglobin	1998	Oxygen delivery Free radical scavenger	48	5	5	1	0	0	0	0	0	0	0	5
Oxygengated flurocarbon nutrient emulsion (OFNE)	2001	Oxygen delivery	94	1	2	0	0	3	0	0	0	0	0	5
Hyperbaric oxygen treatment	1966	Oxygen delivery	24	17	13	5	2	7	4	0	0	0	0	7
Other														
Caffeinol	2002	Stimulant, depressant, diuretic Adenosine receptor modulator	51	10	8	2	0	0	0	0	0	0	0	4
Corticotrophin	1987	GABA receptor modulator Pituitary hormone	—	0	0	0	0	0	0	0	0	0	0	0
Glyceryl trinitrate (nitroglycerin, GTN)	1999	NO donor	—	0	1	0	0	0	0	0	1	0	1	0
Hypothermia	1998	Reduce reducing cerebral oxygen demand (CMRO2), Metabolic and synaptic transmission inhibitor.	46	92	94	28	0	77	28	0	13	3	1	10
ONO-2506	2003	Astrocyte modulating agent Anenuates extracellular monamine	25	8	5	3	0	0	0	0	0	0	0	5
Radix salviae miltiorrhizae	2000	Antioxidant Partial endothelin-1 inhibitor Increases VIP	—	0	1	1	0	1	0	0	1	0	0	1
Repinotan (BAY × 3072)	2000	Serotonin agonist	56	2	2	0	0	2	0	0	7	0	0	2
Simvastatin	2001	HMGCoA reductase inhibitor Antioxidant	30	20	11	1	0	3	1	0	1	0	0	7
TAK-218	2001	Dopamine suppressor Sodium channel modulator	10	1	0	1	0	0	0	0	1	0	0	1
Tinofedrine (D 8955, Novocebrin)	1978	Blood flow, increased metabolism	—	0	0	0	0	0	0	0	0	0	0	0
Trazodone (Desyrel)	1986	Serotonin reuptake inhibitor	—	0	0	0	0	0	0	0	0	0	0	0

[a]First trial = the year in which the drug was first reported to have been given to acute stroke patients.
[b]Level of protection = average neuroprotection from infarct volume changes in focal ischemia studies.
[c]N = number of studies from which the level of neuroprotection has been calculated
[d]+ = number of experimental contrasts with a significant improvement in outcome in the treatment group vs control; 0 = number of experimental contrasts showing no significant in outcome in the treatment group vs control; − = Number of experimental contrasts showing a worse outcome in the treatment group vs control.
[e]STAIR quality = number of STAIR criteria met by the drug (maximum 10: see Table 3).
[f]Analyzed with other group because of low numbers.

No particular drug mechanism distinguished itself on the basis of superior efficacy in animal models of focal ischemia. This may reflect the multifaceted nature of the sequelae of ischemic stroke and suggest a role for combination therapy to target multiple processes. Alternately, it might suggest that our conception of stroke needs reformulation. A tendency to exclusively frame drug activity in terms of the dominant schema of stroke damage (eg. excitotoxicity, free radical damage), coupled with the sometimes arbitrary attribution of a drug mechanism to one of several nonmutually exclusive groupings, might distract from other paradigms with greater explanatory power, thus hindering the development of more effective treatments.

Limitations: Identification of Relevant Data

The search strategy used was broad but not deep. For instance, we have identified 19 publications describing the efficacy of FK506 and 46 for recombinant t-PA, compared with 28 and 94 identified through more systematic methods[9] (also M. R. Macleod and colleagues, unpublished observations). Systematic review then may be combined with meta-analysis to give a more complete description of the efficacy, and limits to efficacy, for individual drugs.[8,26] There is also evidence, for at least some neuroprotectants, of a significant publication bias in favor of positive results (M.R. Macleod, unpublished observations), perhaps for commercial or other reasons. Furthermore, a strong emphasis was

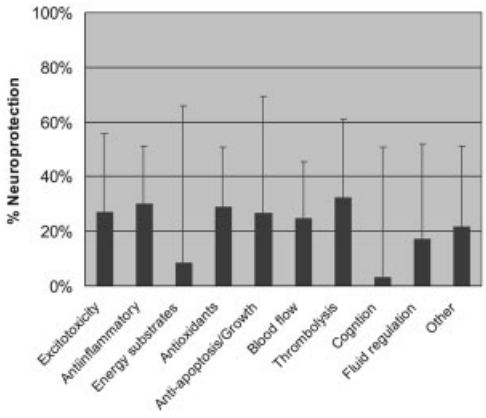

Fig 3. Average neuroprotection in focal models of ischemia for interventions, classified by their putative primary mode of action (100% = complete protection, 0% = no difference from the control condition).

placed on the identification and extraction of data from focal ischemia models because they tend to be considered a better model of human stroke.[11]

Limitations: Changing Experimental Standards

The scale devised for scope of testing was based on the STAIR recommendations for preclinical neuroprotective and restorative drug development. These recommendations were chosen as the gold standard as they represent a crystallization of recent thought on animal experimentation in stroke. Nevertheless, the reasonableness of assessing past experiments using today's standards may be questioned when considerable progress has been made in our understanding and interpretation of animal models of stroke. Researchers' conception of the theoretical underpinnings of stroke models has undergone much refinement over the past decade, and the standards for testing neuroprotective efficacy have been raised accordingly. Furthermore, the STAIR standards may themselves change as more knowledge is gained on the ability of particular models to predict clinical outcome.

Limitations: Drug Mechanism

Each drug was assigned a primary hypothesized mechanism of action. These classes were drawn broadly and may obscure the complex and multifaceted nature of stroke pathophysiology. Such categorization needs much refinement.

Limitations: End Points

The conclusions rest upon the validity of infarct volume as a measure of stroke damage. To the extent that different brain areas are specialized for the performance of different tasks, that recovery of brain function is plastic and that interference with learning and memory is not proportional to the amount of tissue damaged, then infarct volume is compromised as a measure of damage.

Behavioral outcomes and neurological assessments were not included in the quantitative aspects of this review. Assessment and interpretation of animal behavior presents additional challenges in developing stroke models. For instance, because of the difficulties in quantifying responses to tasks, acute assessment scales for rats tend to rely heavily on motor effects in contrast with clinical stroke scales. Even when nonmotor systems are tested, the interpretation of the results must have regard to potential differences between animals and humans in terms of the importance of different modalities, for example, olfaction and whisker movement. For these and other reasons, animal studies tend to place a heavy reliance upon infarct volume compared with functional outcome measures more commonly used in the clinical context.[2] Nevertheless, it is recommended that preclinical studies use both functional and histological outcomes.[11] Challenges in the interpretation of behavioral outcomes are no greater than those encountered in understanding histological end points in stroke models (eg, comparative differences in gray/white matter balance, frontal lobe volume). Inclusion of behavioral outcomes in the review, as we have been doing in meta-analyses,[8,9,26] can only assist in the understanding of experiments.

Limitations: Model Validation

A good animal model should be both reliable and valid, that is, produce consistent, replicable outcomes, have sound theoretical underpinnings, and have the ability to predict the effect of an intervention on clinical outcome. The paucity of positive results in clinical

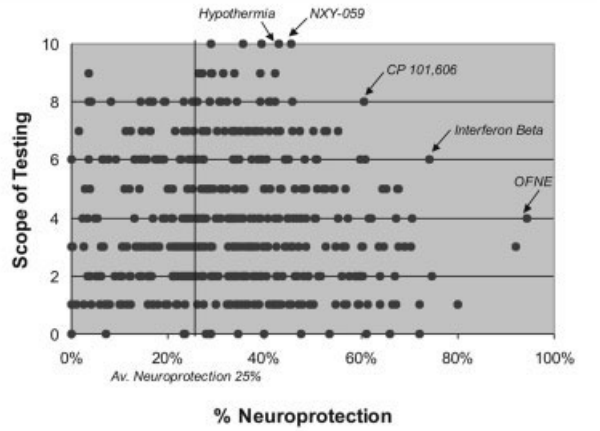

Fig 4. When drugs are tested more extensively in focal models of ischemia (maximum scope of testing = 10), then the average level of neuroprotection tends not to differ greatly from the mean level of protection (25%). OFNE = oxygenated fluorocarbon nutrient emulsion.

trials, together with the disparity in clinical and experimental protocols, have hindered the validation of neurological score and infarct volume in animal experiments as predictors of outcome in human disease. Although animal data would support the use of NXY-059 and t-PA in the clinic, such data might also provide tentative support for the clinical application of many of the other 114 therapies that have been tested in humans but not undergone further clinical development.

Limitations: Model Specificity

Different animal models may be needed to reflect differences in patients with acute ischemic stroke concerning stroke subtype, reperfusion status, and comorbidities. Averaging across all results might mask the potential utility of a treatment within a particular subclass of patients. For instance, thrombolytics such as t-PA tend to produce a better outcome in embolic models of stroke compared with thread occlusion models.[27] Techniques such as meta-analysis and regression modeling are better suited to unraveling the complexity of animal data.[1,8]

Future Directions

More accurate estimates of neuroprotective efficacy will be afforded through systematic review and meta-analyses for individual drugs, and this is now the focus of the Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Stroke, the CAMARADES group. Meta-analysis and regression modeling of pooled data for different drugs may also help establish whether there is indeed a "baseline" positive efficacy in such studies. Further, these techniques will help elucidate the determinants of efficacy in animal models of stroke.

Conclusion

Stroke continues to kill 5.5 million people each year,[28] and the development of safe and effective treatments is a major challenge to experimental and clinical neuroscience. The systematic review and analysis of data from neuroprotection experiments may bring us closer to achieving this goal. It has been suggested that drugs should be taken forward to clinical trial only if data from animal experiments are valid and precise and in the public domain before clinical trials occur[1]: this review affirms that position. Drugs taken forward to clinical trial in the past have not been distinguished by superior efficacy in animal models, and when assessing experimental efficacy attention must also be given to magnitude and scope of preclinical testing. Together with greater rigor in the conduct and reporting of animal data, there is every prospect that such an approach will improve the transition of scientific advances from bench to bedside.

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