

H. Bart van der Worp
Peter de Haan
Erik Morrema
Cor J. Kalkman

Methodological quality of animal studies on neuroprotection in focal cerebral ischaemia

Received: 17 June 2004
Received in revised form:
22 November 2004
Accepted: 4 January 2005

H. B. van der Worp, MD, PhD (✉)
Dept. of Neurology, HP G 03.228
University Medical Centre Utrecht
P. O. Box 85500
3508 GA Utrecht, The Netherlands
Tel.: +31-30/2509111
Fax: +31-30/2542100
E-Mail: h.b.vanderworp@neuro.azu.nl

P. de Haan, MD, PhD
Dept. of Anaesthesiology
Onze Lieve Vrouwe Gasthuis
Amsterdam, The Netherlands

E. Morrema · C. J. Kalkman, MD, PhD
Dept. of Anaesthesiology
University Medical Centre Utrecht
Utrecht, The Netherlands

■ **Abstract** *Background* The recurrent failure of apparently promising neuroprotective drugs to improve outcome in trials of patients with acute ischaemic stroke may partially be explained by overoptimistic conclusions about efficacy as a result of methodological shortcomings in preclinical studies. We assessed the methodological quality of animal studies of five different neuroprotective agents that have been tested in 21 clinical trials including a total of more than 12,000 patients with acute ischaemic stroke. *Methods* We performed a literature search restricted to full publications on the effects of clomethiazole, gavestinel, lubeluzole, selfotel, or tirilazad mesylate on infarct volume or functional outcome in animal models of acute focal cerebral ischaemia. We used a rating scale to assess the methodological quality of the included studies. One point was attributed to each of 10 items. A score of 4 to 6 points was considered “medium” and a score above 7

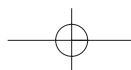
“high.” *Results* A total of 45 articles were included. The median score on the methodological quality index was 3; 18 studies had a medium score and one a high score. Randomised treatment allocation was mentioned in 19 studies (42%), blinded administration of study medication in 10 (22%), and blinded outcome assessment in 18 (40%). The study drug was administered at a median of 10 min (range, –60 to 360 min) after the onset of ischaemia. *Conclusion* The evidence for neuroprotective efficacy that formed the basis for initiating the 21 trials was obtained in animal studies with a methodological quality that would, in retrospect, not justify such a decision. More rigorous preclinical study methodology may lead to more reliable and reproducible results.

■ **Key words** animal models · cerebral ischaemia · neuroprotection · review · methodological quality

Introduction

Although numerous compounds have proved to be effective in animal models of focal cerebral ischaemia, only recombinant-tissue plasminogen activator (rt-PA) and aspirin have convincingly demonstrated efficacy in clinical trials of acute ischaemic stroke [4, 17, 34]. Fac-

tors that may explain the disparity between the results of animal models and clinical trials have been given exhaustive attention in reviews and comments [6, 11–13, 21, 24, 31, 32, 36], and include differences in time windows for drug administration, selection of outcome measures, timing of outcome measurements, and characteristics of the study population other than species. However, the majority of these reviews were based on



the authors' impressions of the characteristics of animal studies rather than on systematic evaluation.

We hypothesised that the recurrent failure of apparently promising neuroprotective drugs to improve functional outcome in patients with acute ischaemic stroke might in part have been caused by inadequate data and overoptimistic conclusions about efficacy as a result of methodological flaws in preclinical studies. This has been suggested before by Wardlaw et al. after the publication of the negative results of the Glycine Antagonist In Neuroprotection (GAIN) trials [36]. To answer this question, we evaluated the methodological quality of animal studies of five different compounds from different classes of alleged neuroprotective agents that have recently been tested in 21 clinical trials including a total of more than 12,000 patients with acute ischaemic stroke [2, 10, 15, 27].

Methods

■ Search Strategy

The literature search for this review was restricted to full publications on the effects of the compounds clomethiazole (a GABA agonist), gavestinel (a glycine site antagonist), lubeluzole (several mechanisms), selfotel (an NMDA antagonist), or tirilazad mesylate (a radical scavenger) on infarct volume or functional outcome in animal models of acute focal cerebral ischaemia. These drugs were chosen because each compound had been tested in at least two phase III clinical trials that included the largest number of patients in their drug category. Publications were identified independently by the first and third author searching Medline (1966 to 2002) and Embase (1980 to 2002) using the names and synonyms of the above compounds, and the key terms [*<stroke> OR <ischaemia> OR <ischemia>*], as described by Macleod et al. [25]. Of all studies thus found, including those in which the results of the clinical trials were presented, reference lists were checked for additional studies. This method of cross-checking was continued until no further studies were found.

■ Eligibility

Criteria for inclusion of studies in this review were (1) assessment of the effect of one or more of the above compounds on infarct volume (or a derivative) and/or functional outcome after focal cerebral ischaemia, (2) description of a control group, (3) journal publication in full. Studies were excluded if the effect of a compound was tested only in combination with another potentially neuroprotective strategy or agent, except for thrombolysis.

■ Data extraction

The first three authors independently extracted data from eligible studies by means of a standardised data extraction form. In case of disagreement, the observers reviewed the article in question together. Data in the following categories were extracted: (1) animal (species, weight, age, gender, and the presence of hypertension or diabetes), (2) model (permanent or temporary ischaemia, and measurements of blood pressure and brain and body temperature), (3) methodology (power calculations, numbers of animals in actively treated and control groups, numbers of excluded animals and reasons for exclusion, method of treatment allocation, blinding of drug administration and

outcome evaluation), (4) drug (name and start time), (5) outcome (type, time of evaluation), and (6) study funding. If outcome was assessed at different time points following the onset of ischaemia in the same animal, only the last assessment was included in the analysis.

■ Methodological quality

A rating scale was used to assess the methodological quality of the included studies (Table 1), based on the "recommendations for standards regarding preclinical and restorative drug development" by the Stroke Therapy Academic Industry Roundtable (STAIR) [31]. This scale resembles that used by Horn et al. [16] in a systematic review of nimodipine in experimental focal cerebral ischaemia. A "clinically relevant time window for start of treatment" was defined as treatment started more than 60 minutes after the onset of ischaemia, as treatment of patients between 60 and 90 minutes after the onset of symptoms has been shown to be feasible in a small group of patients [34]. One point was attributed for each of the 10 items if mentioned in the article. A total score of 0 to 3 was considered "low", a total score of 4 to 6 "medium", and a total score above 7 "high".

■ Statistical analysis

The data were entered on paper review sheets by the individual assessors and entered in a Microsoft Access database after resolution of possible discrepancies between reviewers. Descriptive data are expressed as frequencies, means, or medians and range as appropriate. For two-group comparisons the Mann-Whitney U-test was used.

Results

Electronic searching identified 673 publications, of which 47 (7%) fulfilled the inclusion criteria. Hand-searching identified one additional publication. Two duplicate publications on selfotel were not included. One publication giving an overview of the preclinical development of gavestinel was excluded because the data on the focal cerebral ischaemia experiments were deemed too scanty. Therefore, a total of 45 publications were included in the present study: 9 on the effect of clomethiazole, 4 on gavestinel, 6 on lubeluzole, 10 on selfotel, and 18 on tirilazad mesylate (see appendix). One study tested the effect of both clomethiazole,

Table 1 Methodological quality index (one point for each of the following study attributes)

- Monitoring of physiological parameters
- Group size based on a-priori power calculation
- Treatment allocation via randomisation
- Blinded drug administration
- Blinded outcome evaluation
- Use of aged, diabetic, or hypertensive animals
- Clinically relevant time window for start of treatment
- Assessment of both infarct volume and functional outcome
- Outcome assessment in the acute phase (1 to 6 days)
- Outcome assessment in the chronic phase (7 to 30 days)

gavestinel, and selfotel. Characteristics of the included articles are presented in Table 2. Thirty nine studies (87%) were performed in rodents (29 in rats, 2 in gerbils, 4 in mice, and 5 in rabbits; one study used both rats and mice); we found 3 studies performed in primates and 3 performed in cats. Except in two studies performed in marmosets, the age of the animals was not mentioned. The weight of the rats was almost invariably 250–400 g, indicating an age of less than one-sixth of their normal life expectancy. In 38 studies (84%) only male animals were used, in one (2%) only females, in two (4%) both males and females, and in four (9%) gender was not mentioned. Three studies (7%) were performed in animals with hypertension, and none in animals with diabetes.

In only one study (post-hoc) were power calculations performed. Randomised treatment allocation was mentioned in 19 studies (42%), blinded administration of study medication in 10 (22%), and blinded outcome assessment in 18 (40%). In two articles double blinding for treatment allocation was reported. Exclusion of animals, mainly because of mortality, was mentioned in 12 articles (27%). None of studies used an intention-to-treat analysis. The median score on the methodological quality index was 3 (range, 1 to 7); 26 studies (58%) had a low score, 18 (40%) had a medium score, and one (2%) had a high score.

We derived and analysed a total of 129 individual pairwise comparisons (study drug versus control) from the 45 publications (Table 2). The median group size of actively treated animals was 9, that of control animals 10 (range, 4 to 23 and 5 to 50, respectively). The study drug was administered at a median of 10 min (range, –60 to 360 min) after the onset of ischaemia, and outcome was assessed at a median of 24 h (range, 4h – 20 weeks) after the onset of ischaemia. In 34 of the 129 comparisons

(26%) the time interval between onset of ischaemia and start of treatment was more than one hour. In 29 studies (64%) the manufacturer of the compound under study was involved either financially or in person. There was no relation between involvement of the manufacturer of the compound and methodological quality of the study ($P = 0.95$).

Discussion

The present study confirms previously expressed concerns about the disparity between animal models of focal cerebral ischaemia and clinical trials of acute ischaemic stroke [6, 11–13, 21, 24, 31, 32, 36]. In contrast to the clinical trials of the compounds under study, the animal studies that formed the justification for these trials were often characterised by a short and clinically unattainable time window for start of treatment, very early assessments of outcome, and an emphasis on infarct volume rather than functional outcome as a primary outcome measure. In addition, the characteristics of the experimental animals used did not reflect the population of patients with acute ischaemic stroke. Almost invariably, the animals were young, and were neither hyperglycaemic or hypertensive, conditions that are present in about half and the majority of the patients with acute stroke, respectively [35].

As suggested after the publication of the negative results of the Glycine Antagonist In Neuroprotection (GAIN) International trial [22], methodological flaws observed in the present review may be a fundamental source of bias in the preclinical evaluation of neuroprotective agents [36]. Random treatment allocation was reported in only 42% of the studies reviewed, blinded administration of the study agent in 22%, and blinded

Table 2 Characteristics of the studies

	total	clomethiazole	gavestinel	lubeluzole	selfotel	tirilazad
articles (n) ^a	45	9	4	6	10	18
pair-wise comparisons (n)	129	15	17	49	16	32
total actively treated animals (n)	1265	135	132	555	125	318
total controls (n)	881	97	54	343	122	265
group size active treatment (median (range))	9 (4–23)	9 (4–10)	9 (5–10)	10 (6–23)	8 (5–12)	10 (5–19)
group size controls (median (range))	10 (5–50)	10 (5–10)	9 (7–10)	10 (6–50)	10.5 (5–19)	10 (5–23)
score methodological QI (median (range))	3 (1–7)	2 (1–4)	2 (1–4)	4 (1–5)	3 (1–4)	4 (2–7)
start treatment ^b (minutes, median (range))	10 (–60–360)	60 (–60–180)	60 (–30–360)	30 (0–360)	0 (–30–75)	2.5 (–30–240)
time outcome assessment ^c (h, median (range))	24 (4–3360)	24 (24–3360)	24 (24–144)	24 (4–336)	36 (4–144)	25.5 (4–336)
functional outcome (n (%)) ^d	12 (27)	2 (22)	0 (0)	1 (17)	1 (10)	8 (44)
funding by manufacturer (n (%))	29 (64)	8 (89)	3 (75)	4 (67)	5 (50)	9 (50)

QI indicates quality index; IQR interquartile range

^a numbers do not add up to 45 because one study tested both clomethiazole, gavestinel, and selfotel; ^b in minutes after onset of ischaemia; ^c in hours after onset of ischaemia;

^d number of studies in which functional outcome was tested

assessment of outcome in 40%. Previous evaluations of clinical trials and animal studies have suggested that both non-random and inadequately concealed treatment allocation may lead to overestimation of treatment effects [3, 18, 29]. Negative preclinical studies are much more likely to remain unpublished than negative large clinical trials [7]. In a systematic review of experimental stroke studies describing the efficacy of nicotinamide, comparisons published only in abstract form gave a significantly lower estimate of effect size than those published in full, demonstrating publication bias [25]. It is therefore conceivable that the career of a preclinical investigator is more dependent on obtaining positive results than that of a clinical trialist. For this reason, randomisation and blinding, which are considered essential precautions against bias in clinical trials [26, 30], should be valued equally in animal studies.

Because of their complexity, stroke models are inherently vulnerable to complications that may affect outcome, such as failure to obtain sufficient ischaemia, or perioperative hypotension or even hypoxaemia, when the airway is not secured and the animal is not ventilated with blood gas control. Given the explanatory character of preclinical studies, it appears justifiable to exclude animals with complications from the analyses of treatment effects, provided that the exclusion criteria are predefined and not determined on a *post-hoc* basis, the latter also because of the open character of most experiments. In view of the above, it is not surprising that in none of the studies an intention-to-treat analysis was used. However, only one study mentioned predefined in- and exclusion criteria, and in 12 articles (27%) exclusion of animals from analysis was mentioned and substantiated.

The above factors contributed to the low median score on the employed methodological quality scale. The scale we used differed in several aspects from those used by Horn et al. [16] and Macleod et al. [25]. Horn et al. did not include the items of sample size calculation and blinded administration of the study agent, which we consider essential in the preclinical evaluation of neuroprotectants. Omitting a sample size calculation may lead to a lack of power and thereby to an inability to detect a clinically relevant effect [8]. As discussed above, open administration of the study agent may give rise to a bias in the severity of the induced infarct.

In their 10-point scale, Macleod et al. also attributed points for the following items: peer-reviewed publication; use of an anaesthetic without significant neuroprotective activity (i. e. no ketamine); compliance with animal welfare regulations; and statement of potential conflict of interest [25]. Because of our search strategy, in which abstracts were purposely excluded, we did not attribute points for peer review of the publication. We did not include an item on the anaesthetic used, as not only ketamine [5], but also other frequently used anaes-

thetics such as halothane and isoflurane are reported to have intrinsic neuroprotective properties [14, 19]. Most journals now require a statement of compliance with animal welfare regulations. Although we believe such compliance is a prerequisite for doing animal research, we are not aware of evidence that this improves the methodological quality of experimental studies.

We agree with Macleod et al. that financial interests of authors or sponsors may lead to biased data interpretation. However, as stated above, obtaining positive rather than negative results may not only favour financial interests but also career opportunities. For this reason, we put an emphasis on randomisation and blinding in our quality scale, as means to prevent biased data collection and interpretation.

The last four items of our methodological quality scale, which were all based on the STAIR recommendations [31], were also incorporated in Horn's scale but not in Macleod's. In clinical trials targeting acute ischaemic stroke published between 1995 and 1999, the median time to start of treatment was 14 hours [20]. Despite increasing public awareness of acute stroke treatment, the vast majority of stroke patients do not reach the hospital within 3 hours after the onset of symptoms [1]. Although time windows for effective stroke treatment in rats may not be comparable to those in man, we think it is essential that putative neuroprotective treatment strategies are tested at clinically relevant time points after the onset of ischaemia. In our scale, a "clinically relevant time window for start of treatment" was defined as treatment started more than 60 minutes after the onset of ischaemia, as treatment of patients between 60 and 90 minutes after the onset of symptoms has been shown feasible in a small group of patients [34].

Functional outcome is indisputedly the primary measure of efficacy in clinical trials, whereas animal studies usually rely on infarct volume. Unfortunately, infarct volume does not tell us whether surviving neurons are functional, dysfunctional, or destined for death in a delayed fashion [11]. In addition, several studies have suggested that in patients the relationship between infarct volume and functional outcome is moderate at best [28, 33]. For these reasons, we suggest that in animal studies testing putative neuroprotective compounds both infarct volume and functional outcome should be assessed. We included the items on timing of outcome assessment in our scale because several studies have suggested that some neuroprotective treatment strategies only delay but do not prevent cell death [11]. However, compared with the requirements of clinical trials, our scale is still rather crude and turns a blind eye to several items that are considered desirable in clinical trials. We therefore support the call for the development of more sophisticated quality scores, perhaps with weighting of different components [25].

The aim of our study was to evaluate the method-

ological quality of animal studies testing neuroprotective compounds in focal cerebral ischaemia. We purposely only included full publications and no abstracts. Owing to space constraints, study quality reported in abstracts only is likely to under-represent true study quality. We decided not to perform a meta-analysis of the efficacy results of the animal experiments under study, as this should have included results published only in an abstract.

For the above reason, a drawback of the present study is the fact that we have likely reviewed only a subset of all animal experiments actually performed with the compounds under study. We have limited this review to studies published as full papers and have not included unpublished work, for example preliminary studies performed by the manufacturer in its research laboratories. As there is no registry of animal studies of neuroprotectants in acute ischaemic stroke and unpublished experiments may have been performed in laboratories unknown to us, we did not attempt to obtain unpublished material. Nonetheless, we consider it unlikely that the results of the present review would have been more positive if unpublished studies had been included. We also acknowledge that a decision to start a clinical trial of a specific compound is not only based on published animal work, but also on circumstantial evidence of efficacy from *in vitro* studies and evidence from 'non-stroke' models such as global ischemia and traumatic brain injury. However, the gap between ischaemic neurons in culture and the patient with ischaemic stroke should be bridged by methodologically sound animal studies of focal cerebral ischaemia.

It is obvious that the failure of neuroprotective stroke trials cannot only be attributed to flaws in preclinical studies, but also to shortcomings of clinical trials. These include, amongst others, long time windows for start of treatment, insufficient statistical power, and patient heterogeneity [11, 13]. Several recent publications have provided valuable tools to improve the identification of neuroprotective treatments in clinical trials [9, 23, 32, 37].

In conclusion, the evidence that formed the basis for the decisions that initiated 21 randomised clinical trials including more than 12,000 patients with acute ischaemic stroke was thin and obtained in animal studies with a methodological quality that would not justify a decision to perform these trials. More rigorous pre-clinical study methodology may lead to more reliable and reproducible results.

■ **Acknowledgement** H.B. van der Worp was supported by a grant from the Jan Meerwaldt Foundation.

Appendix

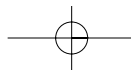
Included publications on clomethiazole [4, 11, 18, 19, 35–39], gavestinel [6, 11, 14, 30], lubeluzole [2, 3, 8, 10, 12, 13], selfotel [11, 17, 21, 22, 24, 28, 29, 31, 34, 41], and tirilazad mesylate [1, 5, 7, 9, 15, 16, 20, 23, 25–27, 32, 33, 40, 42–45].

- Alessandri B, Basciani R, Langemann H, Lyrer P, Pluess D, Landolt H, Gratz O (2000) Chronic effects of an aminosteroid on microdialytically measured parameters after experimental middle cerebral artery occlusion in the rat. *J Clin Neurosci* 7:47–51
- Aronowski J, Strong R, Grotta JC (1996) Combined neuroprotection and reperfusion therapy for stroke. Effect of lubeluzole and diaspirin cross-linked hemoglobin in experimental focal ischemia. *Stroke* 27:1571–1576
- Aronowski J, Strong R, Grotta JC (1996) Treatment of experimental focal ischemia in rats with lubeluzole. *Neuropharmacology* 35:689–693
- Baldwin HA, Williams JL, Snares M, Ferreira T, Cross AJ, Green AR (1994) Attenuation by chlormethiazole administration of the rise in extracellular amino acids following focal ischaemia in the cerebral cortex of the rat. *Br J Pharmacol* 112:188–194
- Beck T, Bielenberg GW (1991) The effect of two 21-aminosteroids on overt infarct size 48 hours after middle cerebral artery occlusion in the rat. *Brain Res* 560:159–162
- Bordi F, Pietra C, Ziviani L, Reggiani A (1997) The glycine antagonist GV150526 protects somatosensory evoked potentials and reduces the infarct area in the MCAo model of focal ischemia in the rat. *Exp Neurol* 145:425–433
- Brown CM, Calder C, Linton C, Small C, Kenny BA, Spedding M, Patmore L (1995) Neuroprotective properties of lifarizine compared with those of other agents in a mouse model of focal cerebral ischaemia. *Br J Pharmacol* 115:1425–1432
- Buchkremer-Ratzmann I, Witte OW (1997) Pharmacological reduction of electrophysiological diaschisis after photothrombotic ischemia in rat neocortex. *Eur J Pharmacol* 320:103–109
- Clark WM, Hotan T, Lauten JD, Coull BM (1994) Therapeutic efficacy of tirilazad in experimental multiple cerebral emboli: a randomized, controlled trial. *Crit Care Med* 22:1161–1166
- Culmsee C, Junker V, Wolz P, Semkova I, Kriegelstein J (1998) Lubeluzole protects hippocampal neurons from excitotoxicity in vitro and reduces brain damage caused by ischemia. *Eur J Pharmacol* 342:193–201
- Dawson DA, Wadsworth G, Palmer AM (2001) A comparative assessment of the efficacy and side-effect liability of neuroprotective compounds in experimental stroke. *Brain Res* 892:344–350
- De Ryck M, Keersmaekers R, Duytschaever H, Claes C, Clincke G, Janssen M, Van Reet G (1996) Lubeluzole protects sensorimotor function and reduces infarct size in a photochemical stroke model in rats. *J Pharmacol Exp Ther* 279:748–758
- De Ryck M, Verhoye M, Van der Linden AM (2000) Diffusion-weighted MRI of infarct growth in a rat photochemical stroke model: effect of lubeluzole. *Neuropharmacology* 39:691–702
- Di Fabio R, Conti N, De Magistris E, Feriani A, Provera S, Sabbatini FM, Reggiani A, Rovatti L, Barnaby RJ (1999) Substituted analogues of GV150526 as potent glycine binding site antagonists in animal models of cerebral ischemia. *J Med Chem* 42:3486–3493
- Hall ED, Pazara KE, Braughler JM (1988) 21-aminosteroid lipid peroxidation inhibitor U74006F protects against cerebral ischemia in gerbils. *Stroke* 19:997–1002
- Hellstrom HO, Wanhainen A, Valtysson J, Persson L, Hillered L (1994) Effect of tirilazad mesylate given after permanent middle cerebral artery occlusion in rat. *Acta Neurochir (Wien)* 129:188–192
- Jolkkonen J, Puurunen K, Koistinaho J, Kauppinen R, Haapalinna A, Nieminen L, Sivenius J (1999) Neuroprotection by the alpha2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *Eur J Pharmacol* 372:31–36
- Marshall JW, Cross AJ, Jackson DM, Green AR, Baker HF, Ridley RM (2000) Clomethiazole protects against hemineglect in a primate model of stroke. *Brain Res Bull* 52:21–29
- Marshall JW, Cross AJ, Ridley RM (1999) Functional benefit from clomethiazole treatment after focal cerebral ischemia in a nonhuman primate species. *Exp Neurol* 156:121–129
- Meden P, Overgaard K, Pedersen H, Boysen G (1996) Effect of early treatment

- with tirilazad (U74006F) combined with delayed thrombolytic therapy in rat embolic stroke. *Cerebrovasc Dis* 6:141–148
21. Minger SL, Geddes JW, Holtz ML, Craddock SD, Whiteheart SW, Siman RG, Pettigrew LC (1998) Glutamate receptor antagonists inhibit calpain-mediated cytoskeletal proteolysis in focal cerebral ischemia. *Brain Res* 810:181–199
 22. Miyabe M, Kirsch JR, Nishikawa T, Koehler RC, Traystman RJ (1997) Comparative analysis of brain protection by N-methyl-D-aspartate receptor antagonists after transient focal ischemia in cats. *Crit Care Med* 25:1037–1043
 23. Mori E, Ember J, Copeland BR, Thomas WS, Koziol JA, del Zoppo GJ (1995) Effect of tirilazad mesylate on middle cerebral artery occlusion/reperfusion in nonhuman primates. *Cerebrovasc Dis* 5:342–349
 24. Okada M, Ueda H, Kometani M, Nakao K (1997) Effect of D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid on focal cerebral ischemia in cat. *Arzneimittelforschung* 47:703–705
 25. Oktem IS, Menku A, Akdemir H, Kontas O, Kurtsoy A, Koc RK (2000) Therapeutic effect of tirilazad mesylate (U-74006F), mannitol, and their combination on experimental ischemia. *Res Exp Med (Berl)* 199:231–242
 26. Orozco J, Mendel RC, Hahn MR, Guthkelch AN, Carter LP (1995) Influence of a 'brain protector' drug 21-amino steroid on the effects of experimental embolic stroke treated by thrombolysis. *Neurol Res* 17:423–425
 27. Park CK, Hall ED (1994) Dose-response analysis of the effect of 21-amino-steroid tirilazad mesylate (U-74006F) upon neurological outcome and ischemic brain damage in permanent focal cerebral ischemia. *Brain Res* 645:157–163
 28. Perez-Pinzon MA, Maier CM, Yoon EJ, Sun GH, Giffard RG, Steinberg GK (1995) Correlation of CGS 19755 neuroprotection against in vitro excitotoxicity and focal cerebral ischemia. *J Cereb Blood Flow Metab* 15:865–876
 29. Poinet H, Nowicki JP, Scatton B (1992) Lack of neuroprotective effect of some sigma ligands in a model of focal cerebral ischemia in the mouse. *Brain Res* 596:320–324
 30. Reggiani A, Pietra C, Arban R, Marzola P, Guerrini U, Ziviani L, Boicelli A, Sbarbati A, Osculati F (2001) The neuroprotective activity of the glycine receptor antagonist GW150526: an in vivo study by magnetic resonance imaging. *Eur J Pharmacol* 419:147–153
 31. Sauer D, Allegrini PR, Cosenti A, Pataki A, Amacker H, Fagg GE (1993) Characterization of the cerebroprotective efficacy of the competitive NMDA receptor antagonist CGP40116 in a rat model of focal cerebral ischemia: an in vivo magnetic resonance imaging study. *J Cereb Blood Flow Metab* 13:595–602
 32. Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ (1998) Monotherapy with dextromethorphan or tirilazad – but not a combination of both – improves outcome after transient focal cerebral ischemia in rats. *Exp Brain Res* 122:121–127
 33. Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ (1999) Neuroprotective effects of combination therapy with tirilazad and magnesium in rats subjected to reversible focal cerebral ischemia. *Neurosurg* 44:163–171
 34. Simon R, Shiraishi K (1990) N-methyl-D-aspartate antagonist reduces stroke size and regional glucose metabolism. *Ann Neurol* 27:606–611
 35. Snape MF, Baldwin HA, Cross AJ, Green AR (1993) The effects of chlormethiazole and nimodipine on cortical infarct area after focal cerebral ischaemia in the rat. *Neuroscience* 53:837–844
 36. Sydserff SG, Cross AJ, Green AR (1995) The neuroprotective effect of chlormethiazole on ischaemic neuronal damage following permanent middle cerebral artery ischaemia in the rat. *Neurodegeneration* 4:323–328
 37. Sydserff SG, Cross AJ, Murray TK, Jones JA, Green AR (2000) Clomethiazole is neuroprotective in models of global and focal cerebral ischemia when infused at doses producing clinically relevant plasma concentrations. *Brain Res* 862:59–62
 38. Sydserff SG, Cross AJ, West KJ, Green AR (1995) The effect of chlormethiazole on neuronal damage in a model of transient focal ischaemia. *Br J Pharmacol* 114:1631–1635
 39. Sydserff SG, Green AR, Cross AJ (1996) The effect of oedema and tissue swelling on the measurement of neuroprotection; a study using chlormethiazole and permanent middle cerebral artery occlusion in rats. *Neurodegeneration* 5:81–85
 40. Takeshima R, Kirsch JR, Koehler RC, Traystman RJ (1994) Tirilazad treatment does not decrease early brain injury after transient focal ischemia in cats. *Stroke* 25:670–676
 41. Takizawa S, Hogan M, Hakim AM (1991) The effects of a competitive NMDA receptor antagonist (CGS-19755) on cerebral blood flow and pH in focal ischemia. *J Cereb Blood Flow Metab* 11:786–793
 42. Umemura K, Wada K, Uematsu T, Mizuno A, Nakashima M (1994) Effect of 21-aminosteroid lipid peroxidation inhibitor, U74006F, in the rat middle cerebral artery occlusion model. *Eur J Pharmacol* 251:69–74
 43. Williams LR, Oostveen JA, Hall ED, Jolly RA, Satoh PS, Petry TW (1996) Cyclophosphamide is neuroprotective in a gerbil model of transient severe focal cerebral ischemia: correlation with effects of tirilazad mesylate (U-74006F). *J Neurotrauma* 13:103–113
 44. Wilson JT, Bednar MM, McAuliffe TL, Raymond S, Gross CE (1992) The effect of the 21-aminosteroid U74006F in a rabbit model of thromboembolic stroke. *Neurosurg* 31:929–934
 45. Xue D, Slivka A, Buchan AM (1992) Tirilazad reduces cortical infarction after transient but not permanent focal cerebral ischemia in rats. *Stroke* 23:894–899

References

1. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM (2001) Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. *Neurology* 56:1015–1020
2. Bath PM, Iddenden R, Bath FJ, Or-gogozo J-M, Tirilazad International Steering Committee (2001) Tirilazad for acute ischaemic stroke. *Cochrane Database Syst Rev* CD002087
3. Bebartha V, Luyten D, Heard K (2003) Emergency medicine animal research: Does use of randomization and blinding affect the results? *Acad Emerg Med* 10:684–687
4. CAST (Chinese Acute Stroke Trial) Collaborative Group (1997) CAST: randomised placebo-controlled trial of early aspirin use in 20000 patients with acute ischaemic stroke. *Lancet* 349: 1641–1649
5. Chang ML, Yang J, Kem S, Klaidman L, Sugawara T, Chan PH, Adams JD Jr (2002) Nicotinamide and ketamine reduce infarct volume and DNA fragmentation in rats after brain ischemia and reperfusion. *Neurosci Lett* 322: 137–140
6. Drummond JC, Piyash PM, Kimbro JR (2000) Neuroprotection failure in stroke. *Lancet* 356:1032–1033
7. Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR (1991) Publication bias in clinical research. *Lancet* 337: 867–872
8. Festing MF (2003) Principles: the need for better experimental design. *Trends Pharmacol Sci* 24:341–345
9. Fisher M (2003) Recommendations for advancing development of acute stroke therapies: Stroke Therapy Academic Industry Roundtable 3. *Stroke* 34: 1539–1546
10. Gandolfo C, Sandercock P, Conti M (2002) Lubeluzole for acute ischaemic stroke. *Cochrane Database Syst Rev* CD001924
11. Gladstone DJ, Black SE, Hakim AM, Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery (2002) Toward wisdom from failure. Lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke* 33:2123–2136
12. Grotta J (1995) Why do all drugs work in animals but none in stroke patients? 2 Neuroprotective therapy. *J Intern Med* 237:89–94
13. Grotta J (2001) Neuroprotection is unlikely to be effective in humans using current trial designs. *Stroke* 33: 306–307



14. Haelewyn B, Yvon A, Hanouz JL, MacKenzie ET, Ducouret P, Gerard JL, Roussel S (2003) Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat. *Br J Anaesth* 91:390–396
15. Hankey GJ (2002) Clomethiazole: an unsuccessful bachelor, but perhaps a properous married man? *Stroke* 33: 128–129
16. Horn J, de Haan RJ, Vermeulen M, Luiten PGM, Limburg M (2001) Nimodipine in animal model experiments of focal cerebral ischemia. *Stroke* 32:2433–2438
17. International Stroke Trial Collaborative Group (1997) The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, or both, or neither among 19435 patients with acute ischaemic stroke. *Lancet* 349:1569–1581
18. Ioannidis JPA, Haidich A-B, Pappa M, Pantazis N, Kokori SI, Tektonidou MG, Contopoulos-Ioannidis DG, Lau J (2001) Comparison of evidence of treatment effects in randomized and nonrandomized studies. *JAMA* 286: 821–830
19. Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM (2000) Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. *Anesthesiol* 92: 1335–1342
20. Kidwell CS, Liebeskind DS, Starkman S, Saver JL (2001) Trends in acute ischemic stroke trials through the 20th century. *Stroke* 32:1349–1359
21. Lees KR (2002) Neuroprotection is unlikely to be effective in humans using current trial designs: an opposing view. *Stroke* 33:308–309
22. Lees KR, Asplund K, Carolei A, Davis SM, Diener H-C, Kaste M, Orgogozo J-M, Whitehead J, for the GAIN International Investigators (2000) Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: a randomised controlled trial. *Lancet* 355:1949–1954
23. Lees KR, Hankey GJ, Hacke W (2003) Design of future acute-stroke treatment trials. *Lancet Neurol* 2:54–61
24. Liebeskind DS, Kasner SE (2001) Neuroprotection for ischaemic stroke: an unattainable goal? *CNS Drugs* 15: 165–174
25. Macleod MR, O'Collins T, Howells DW, Donnan GA (2004) Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke* 35:1203–1208
26. Miettinen OS (1983) The need for randomisation in the study of intended effects. *Stat Med* 2:267–271
27. Muir KW, Lees KR (2003) Excitatory amino acid antagonists for acute stroke. *Cochrane Database Syst Rev* CD001244
28. Saver JL, Johnston KC, Homer D, Wityk R, Koroshetz W, Truskowski LL, Haley EC, The RANTTAS Investigators (1999) Infarct volume as a surrogate or auxiliary outcome measure in ischemic stroke clinical trials. *Stroke* 30:293–298
29. Schulz KF, Chalmers I, Hayes RJ, Altman DG (1995) Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA* 273:408–412
30. Schulz KF, Grimes DA (2002) Blinding in randomised trials: hiding who got what. *Lancet* 359:696–700
31. Stroke Therapy Academic Industry Roundtable (STAIR) (1999) Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke* 30:2752–2758
32. Stroke Therapy Academic Industry Roundtable II (STAIR II) (2001) Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke* 32:1598–1606
33. The National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study Group (2000) Effect of intravenous recombinant tissue plasminogen activator on ischemic stroke lesion size measured by computed tomography. *Stroke* 31:2912–2919
34. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (1995) Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 333:1581–1587
35. Van der Worp HB, Kappelle LJ (1998) Complications of acute ischaemic stroke. *Cerebrovasc Dis* 8:124–132
36. Wardlaw JM, Warlow CP, Sandercock PAG, Dennis MS, Lindley RI (2000) Neuroprotection disappointment yet aGAIN. *Lancet* 356:597
37. Weir CJ, Kaste M, Lees KR (2004) Targeting neuroprotection clinical trials to ischemic stroke patients with potential to benefit from therapy. *Stroke* 35:2111–2116

