A Systematic Review and Meta-Analysis of the Efficacy of Piracetam and Piracetam-Like Compounds in Experimental Stroke

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Key Words
Piracetam   Oxiracetam   Levetiracetam   GVS-111   Noopept   Stroke   Ischaemia   Meta-analysis   Systematic review   Neuroprotection

Introduction

More than 1,000 drugs have been tested in experimental stroke, with nearly 500 drugs having reported efficacy in animal models of focal cerebral ischaemia [1]. However, there is to date unequivocal evidence for the clinical efficacy of only 2 drugs – asprin [2] and tissue plasminogen activator [3]. This discrepancy has previously been attributed either to falsely positive animal studies or to biological differences between species which restrict the fidelity with which these animal experiments model human disease. However, a recent analysis comparing animal data describing the efficacy of tissue plasminogen activator with clinical trial data suggests that where the design of a clinical trial matches the conditions under which preclinical efficacy was demonstrated, concordance is observed [4]. It may therefore be possible to explain the poor concordance between animal and clinical data on the basis of falsely negative clinical trials [5].

Our ability accurately to interpret the available animal evidence for the efficacy of candidate drugs is crucial to the process of deciding which therapies should be continued into clinical trial and how they should be tested. We therefore set out to establish the range and quality of the animal data supporting the efficacy of piracetam, a candidate neuroprotective drug tested in clinical trial from 1992 in over 1,000 patients.

Systematic review and meta-analysis allow the methodical collection and interpretation of existing data. Systematic review seeks to identify all relevant studies, thereby minimising selection bias. Meta-analysis com-
and GVS-111
differences between these nootropic drugs to be drawn.

different selectivity in their effects which might suggest
der nootropics simply recapitulate the effects of piracetam
(2-oxo-1-pyrrolidineacetamide) AND ((stroke) OR (ischaemia) OR (cerebrovas-
cular) OR (middle cerebral artery) OR (MCA) OR (ACA) OR (an-
terior cerebral artery) OR (MCAO)) AND (animals) NOT (coronary
OR myocardia*);
(2) hand searching of abstracts of scientific
meetings including the Society for Neuroscience and the Interna-
tional Society for Cerebral Blood Flow and Metabolism; (3) refer-
cences to other studies and unpublished data were requested from
the senior authors of our identified resources.

Inclusion criteria: 2 investigators (P.C.R.W., E.S.S.) independ-
ently assessed the titles and abstracts identified by these search-
and, the findings were collated. Full copies of each of the iden-
tified publications were obtained and those describing controlled
studies of the effect of piracetam or piracetam-like compounds in
animal models of focal cerebral ischaemia where the outcome was
measured as infarct size and/or a neurological score were selected
for further analysis. Disagreements about the selection of studies
were resolved in discussion with a third investigator (M.R.M.).

Methods of review: range and quality of studies – the Stroke
Therapy Academic Industry Roundtable (STAIR) recommenda-
tions [15] relate to both the range and quality of experimental
evidence. We assessed the range of evidence with a modified ver-
ion of the STAIR criteria and determined if the agent had been
tested in: (i) 2 or more laboratories, (ii) 2 or more species, (iii) test-
ing in animals with comorbidities, (iv) male and female animals,
(v) permanent and temporary models of ischaemia, (vi) at least
1 h after vessel occlusion, (vii) at least 2 doses, (viii) a route of drug
delivery that would be feasible in man, (ix) both histological and
neurobehavioural outcomes measured, (x) outcome measured at
least 4 weeks after vessel occlusion (xi) in a larger non-rodent spe-
cies (e.g. primate).

Further to this we assessed study quality against the CAMA-
RADES (Collaborative Approach to Meta-Analysis of Animal
Data from Experimental Stroke) 10-item checklist [16], which
comprises (i) publication in a peer-reviewed journal, (ii) state-
ment of control of temperature, (iii) randomization to treatment
or control, (iv) blinded induction of ischaemia (i.e. concealment
of treatment group allocation at time of induction of ischaemia),
(v) blinded assessment of outcome, (vi) avoidance of anaesthetics
with marked intrinsic neuroprotective properties, (vii) use of an-
wimals with hypertension or diabetes, (viii) sample size calcula-
tion, (ix) statement of compliance with regulatory requirements,
and (x) statement regarding possible conflicts of interest.

Data extraction: a ‘comparison’ was defined as ‘the assessment
of outcome in the treatment and control groups following treat-
ment with a given dose of a drug or vehicle, with treatment com-
mencing at a given time before or after the induction of cerebral
ischaemia [16]. Each comparison was performed by extracting
data for mean outcome, standard deviation and number of ani-
mals per group. Where data were expressed graphically, values
were requested from the authors, and where a response was not
received, values were carefully read from the graphs. Where the
drug was given in multiple doses, the total dose administered in
the first 24 h following induction of ischaemia was recorded. Where
neurological tests were performed serially, the final result
was recorded. We also extracted data for the anaesthetic used, time of outcome measurement, method of induction of ischaemia and the individual component items of the quality checklist described above. Two reviewers independently extracted data and differences were resolved by discussion.

**Meta-analysis:** the data were normalized to outcome in the control group such that complete reversal of the ischaemic injury would give an effect size of 100%. Effect sizes from different experiments were combined using weighted mean difference with a random effects model to give an overall estimate. Where a single control group served multiple treatment groups, the size of the control group was adjusted to account for this. We assessed the effect of a number of variables on effect size by stratifying data according to: drug; drug dosage; time of drug administration; reported quality score; outcome measure; anaesthetic used; route of drug delivery; permanent or temporary ischaemia; method of occlusion; time to outcome measurement; single or multiple dosing regime; and species and gender of animal used. The significance of differences between n groups was assessed by partitioning heterogeneity and using the $\chi^2$ distribution with n − 1 degrees of freedom (d.f.). To allow for multiple comparisons we set our significance level at $p < 0.001$.

## Results

The initial search identified 467 publications. Of these, 7 (6 full publications and 1 abstract) described the efficacy of nootropic drugs in an animal model of focal cerebral ischaemia and expressed the outcome as a volume of infarction or a neurological score. Hand searching did not identify any further studies, and in response to a request for further relevant information 1 author confirmed that data in an abstract were contained within another included publication. Our analysis is therefore based upon data from 6 full publications describing the efficacy of 4 nootropic drugs (piracetam, levetiracetam, oxiracetam and GVS-111) in experimental stroke, published between 1991 and 2003 [17–22]. Within these 6 studies 16 comparisons were identified describing the outcome in 245 animals (table 1). Five publications reported infarct volume (14 comparisons; 197 animals) and 1 reported a neurobehavioural outcome (2 comparisons; 48 animals). The neurobehavioural data were not analyzed further as the data originate from 1 study.

### Study Range and Quality

The efficacy of levetiracetam, oxiracetam and GVS-111 in models of ischaemic stroke was each described in 1 study and 2 studies reported the use of piracetam. There was evidence for efficacy under a range of conditions, meeting 6 of the STAIR criteria. The median reported quality score was 4 (fig. 1; table 2, inter-quartile range = 4–6). No study reported the blinded induction of

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Drug</th>
<th>Species</th>
<th>Total number of animals</th>
<th>Dose range mg/kg</th>
<th>Time of administration, min</th>
<th>Anaesthetic used</th>
<th>Type of ischaemia</th>
<th>Route of delivery</th>
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ischaemia or a sample size calculation and no study included a statement of potential conflict of interest. This is a possible source of bias.

**Efficacy**

Overall, nootropic compounds were reported to reduce the infarct volume by 30.2% (95% CI = 16.1–44.4: $\chi^2 = 47$, d.f. = 13, $p < 10^{-5}$; fig. 2a). There was good evidence for the efficacy of piracetam (48.5% reduction in infarct volume; 95% CI = 34.9–62.1), but for GVS-111 and levetiracetam the 95% CI were wide and no conclusions can be drawn (GVS-111: 51.1%; 95% CI = 11.8–90.5; levetiracetam: 20.3%; 95% CI = 7.6–33.0), and oxiracetam was not effective (–1.8%; 95% CI = −15.5 to 11.8; fig. 2b). The efficacy appeared to be significantly higher in healthy compared with hypertensive animals ($\chi^2 = 27$, d.f. = 13, $p < 0.001$; fig 3a), when the assessment of outcome was blinded ($\chi^2 = 25$, d.f. = 12, $p < 0.001$; fig. 3b) and when allocation to group was random ($\chi^2 = 29$, d.f. = 13, $p < 0.001$; fig. 3c). The reported efficacy was highest when halothane anaesthesia was used ($\chi^2 = 42$, d.f. = 13, $p < 0.001$; fig 3d) and when the outcome was assessed between 2 and 7 days ($\chi^2 = 30$, d.f. = 13, $p < 0.001$; fig. 3e).

There was no significant effect of the route of drug administration, the type of ischaemia, the method of induction of middle cerebral artery occlusion or the time of drug administration (not shown), and there were no data from animals other than male rats.

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**Fig. 1.** Effect of study quality on the estimate of efficacy. The shaded grey bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% CI for the individual estimates. The width of each vertical bar reflects the log of the number of animals contributing to that comparison. Each stratification accounts for a significant proportion of the heterogeneity observed between studies ($p < 0.001$).

**Fig. 2.** a Individual comparisons ranked according to their effect on infarct volume. The shaded grey bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% CI for the individual estimates. ▲ = GVS-111; ● = levetiracetam; ■ = oxiracetam; ○ = piracetam. b Effect of drug used on the estimate of efficacy. The shaded grey bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% CI for the individual estimates. The width of each vertical bar reflects the log of the number of animals contributing to that comparison. There was significant between-study heterogeneity for the analysis of drug used ($\chi^2 = 40$, d.f. = 13, $p < 0.001$).
Fig. 3. Effect of comorbidity (a), blinded assessment of outcome (b), random allocation to group (c), anaesthetic used (d) and delay to outcome assessment on the estimate of efficacy (e). The shaded grey bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% CI for the individual estimates. The width of each vertical bar reflects the log of the number of animals contributing to that comparison. Each stratification accounts for a significant proportion of the heterogeneity observed between studies (p < 0.001).
Discussion

Effects of Piracetam in Animal Models of Stroke

Piracetam and related compounds appear to improve the outcome in animal models of stroke. However, of the 6 studies included in this analysis, only 2 of these related to piracetam [21], and it is difficult to draw robust conclusions from such limited data. The first article was published 10 years after the initiation of the major clinical trial of piracetam in acute stroke [23], suggesting that the decision to progress to clinical trials for piracetam was not based on animal data. It is possible that unpublished data may have been available to those mounting the clinical trial, but it seems unlikely that these data would have remained unpublished for 10 years. A similar observation has been made regarding clinical trials of nimodipine in acute stroke [24]. The failure of clinical trials of both piracetam and nimodipine cannot therefore be taken as a failure of animal modelling of stroke, given that the decision to proceed to clinical trial cannot have been based on published data from animal experiments. Indeed, it may be that had the in vivo efficacy of these compounds been adequately explored (and even now there are insufficient data to allow this), the clinical trials might not have been carried out or they might have been designed differently. For instance, while there are no animal data describing the efficacy of piracetam given more than 6 h after stroke onset, the largest clinical trial allowed a delay to treatment of 12 h.

Limitations of This Analysis

There are limitations to our approach. For many candidate neuroprotective drugs there is evidence for a substantial publication bias such that neutral studies appear to be less likely to be published than those reporting positive findings [25, 26]. With regard to nootropics, there are too few studies reliably to exclude even a major publication bias. If such a bias exists, as seems probable, we may substantially have overstated efficacy. Further, our search strategy may not have identified all relevant publications, particularly as some of these might date from prior to the introduction of electronic biomedical database indexing in the late 1960s.

Biological and systematic sources of bias in individual studies are reflected within the global estimate of outcome, perhaps causing an overstatement of effect size. In contrast to other reviews of neuroprotective drugs [27], higher-quality studies gave higher estimates of efficacy. However, there was an association between study quality and drug (with studies involving piracetam, rather than other nootropics, being of higher quality) and it is plausible that the increased efficacy is due to the drug used and not to study quality. The importance of individual quality scale items in the reduction of biological and systematic bias is therefore not clear and we are exploring this further, in multivariate analysis, across a range of candidate neuroprotective drugs.

While we prespecified the sub-group analysis to be performed and adopted a conservative significance level, some of our findings may have arisen by chance, or to bias or confounding factors as described above, and so these data should be interpreted with caution.

Translation to Clinical Trials

Between 1988 and 1996, over 5,000 stroke patients were randomized in clinical trials of piracetam [23], tirilazad [28] and clomethiazole [29]; each of these studies produced at best a neutral outcome. A Cochrane review of piracetam [30] reported a non-significant 31% (95% CI = –5 to +81%) increase in mortality at 1 month. A post-hoc analysis of data from the Piracetam Acute Stroke Study suggested that piracetam might have a beneficial effect if treatment was initiated within 7 h of stroke onset [23], where indeed there are at least limited animal data supporting efficacy. The importance of concordance in the time to treatment in translating efficacy from animal studies to clinical trial echoes our findings for tirilazad [4]; animal data suggested a substantial efficacy not seen in clinical trial, but the interval between stroke onset and treatment initiation was substantially longer in clinical (median around 5 h) than in animal (median 10 min) studies. The hypothesis that piracetam given soon after stroke onset would be effective is consistent with the available animal data and was tested in the Piracetam in Acute Stroke Study II; unfortunately the results of this study have not been published.

Conclusion

While the overall estimate of efficacy from this meta-analysis suggests that piracetam and piracetam-like compounds have substantial neuroprotective action in models of focal cerebral ischaemia, the data for piracetam are based on only 98 animals, were published at least 10 years after clinical trials had been initiated, and those clinical trials tested efficacy at delays to treatment for which there
were no supporting animal data. The introduction of candidate neuroprotective drugs to clinical trial should be based on a much more rigorous analysis of animal data if these are to be used to justify proceeding to clinical trial.

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References


