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Systematic Review and Meta-Analysis of the Efficacy of Tirilazad in Experimental Stroke

Emily Sena, BSc; Philippa Wheble, BSc; Peter Sandercock, MD; Malcolm Macleod, PhD

Background and Purpose—Tirilazad is a candidate neuroprotective drug with reported efficacy in animal models of stroke that was, however, without benefit in clinical trials. This apparent contradiction might be explained if the animal studies were falsely positive, if the clinical trials were falsely negative, or if tirilazad was not tested under the same conditions in animal and clinical studies. Here we use systematic review and meta-analysis to describe the characteristics and limits to the neuroprotective action of tirilazad in animal models of stroke.

Methods—Systematic review and meta-analysis of studies describing the efficacy of tirilazad in animal models of focal ischemia, in which outcome was measured as infarct volume and/or neurological score. Weighted mean difference random effects meta-analysis was used to measure overall efficacy in prespecified subgroups.

Results—Eighteen studies describing outcome in 544 animals were identified. Study quality (median score, 5/10; interquartile range, 4 to 6) was similar to that seen in systematic reviews of other candidate neuroprotective drugs. Tirilazad reduced infarct volume by 29.2% (95% confidence interval 21.1% to 37.2%) and improved neurobehavioral score by 48.1% (95% confidence interval 29.3% to 66.9%).

Conclusion—Tirilazad may have substantial efficacy in animal models of stroke, but this conclusion must be qualified because of the presence of potential sources of bias. (Stroke. 2007;38:000-000.)

Key Words: meta-analysis ■ neuroprotection ■ stroke ■ systematic review ■ tirilazad

At least 883 candidate thrombolytic and neuroprotective drugs have been tested in animal models of stroke4 and show at least some evidence for efficacy; 97 of these drugs have been tested in human ischemic stroke. To date, there is unequivocal evidence for efficacy for only 2 drugs, aspirin2 and tPa.3 Clinical trials of NXY-0594 are ongoing.

Explaining the discrepancy between efficacy in animal studies and lack of efficacy in clinical trials might lead to important insights and guide the future design of studies of both animal and human strokes.5 There are a number of potential reasons for such differences, including systematic errors in the ways in which data from promising animal studies are used to inform the design of clinical trials. Specifically, the animal data may be falsely positive, reporting a protective effect in which no biological efficacy exists; or the clinical trials may be falsely neutral, reporting no effect when clinical efficacy does in fact exist.

Systematic review of all the available animal evidence reduces selection bias and random error in the assessment of drug efficacy. Useful information can still be extracted even when identified animal studies are too heterogeneous (because of differences in dose, time, species) to provide a reliable estimate of “average” efficacy. Stratified meta-analysis (in which studies are analyzed by dose or time or species) can describe dose-response relationships, time dependence of efficacy, and the impact of study quality and study design characteristics.

These techniques have proved useful in the analysis of data from clinical trials and have been advocated for the analysis of data from animal experiments.5,6 Animal models of stroke lend themselves to this approach, as evidenced by the publication of a growing number of such analyses in recent years.7-13

During ischemia and reperfusion, free radicals play an important role in inducing cerebral injury through effects on DNA, on mitochondria, and through the effects of lipid peroxidation.14 Tirilazad is a synthetic lipid-soluble 21-aminosteroid with antioxidant effects that is proposed to interact with the lipid peroxidation cascade at various stages including: (1) scavenging of hydroxyl and lipid peroxy radicals; (2) maintenance of endogenous antioxidant levels; and (3) prevention of propagation of lipid peroxidation by membrane stabilization.15

Experimental animal data had suggested tirilazad as a treatment for ischemic stroke, but it was subsequently demonstrated to increase death and dependency in a meta-analysis of clinical studies.16 Here we present a systematic review and meta-analysis of the efficacy of tirilazad in experimental stroke. We have set out to establish the quality of the animal studies; the limits (dose response, time dependence) to
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IP indicates intraperitoneal; IV, intravenous.
efficacy; the impact of study characteristics on efficacy; and
to establish the fidelity with which clinical trial design
reflected the conditions under which efficacy was seen in
animals.

Methods

Identification of Relevant Studies
We used: (1) electronic search of Pubmed, EMBASE, BIOSIS using
search terms tirilazad OR U-74006F OR Freedox OR 21-
aminosteroid AND stroke OR ischemia OR cerebrovascular OR
middle cerebral artery AND Animals (Mesh:noexp) OR MCA AND
Animals (Mesh:noexp) OR ACA AND Animals (Mesh:noexp) OR
anterior cerebral artery AND Animals (Mesh:noexp) OR MCAO
AND Animals (Mesh:noexp) AND Animals (Mesh:noexp) NOT
coronary OR myocardia; (2) hand searching of abstracts of 3rd to 5th
World Stroke Conferences, European Stroke Conference (from 2nd
meeting/1992 onwards), International Stroke Conference (from 25th
meeting/2000 onwards; previously in BIOSIS), conferences of the
International Society for Cerebral Blood and Metabolism (from 16th
meeting/1993 onwards); and (3) requests to authors of publications
identified above for other published or unpublished data.

Criteria for Inclusion
Two investigators (E.S., P.W.) independently extracted those publi-
cations identified here that described controlled studies of tirilazad
given in models (whole live animals excluding humans) of focal
cerebral ischemia induced by occlusion of the middle or anterior
cerebral artery or their branches, where tirilazad was administered by
any route and outcome compared with animals receiving placebo or
no tirilazad. Disagreements were resolved in discussion with a third
investigator (M.M.).

End Points Considered
The primary outcome measure was infarct area or volume (deter-
dined histologically or by cross-sectional imaging), with secondary
outcome measures of death and of neurobehavioral score.

Methods of the Review

Quality Assessment
There was no quality threshold for inclusion. Study quality was
assessed against our published ten item checklist6 comprising: (1)
publication in peer reviewed journal; (2) statement of control of
temperature; (3) randomization to treatment or control; (4) blinded
induction of ischemia; (5) blinded assessment of outcome; (6)
avoidance of anesthetics with marked intrinsic neuroprotective
properties; (7) use of animals with hypertension or diabetes; (8)
sample size calculation; (9) statement of compliance with regulatory
requirements; and (10) statement regarding possible conflicts of
interest.

Data Extraction
From each source we identified individual comparisons in which
outcome was measured in a group of animals receiving a specified
dose(s) of drug at a specified time(s) and compared with outcome in
a control group. When the treatment group received more than one
intervention, this was recorded. For each comparison and for each of
treatment and control group, we extracted data for number per group,
mean outcome, and its standard deviation. When an outcome was
measured serially, only the last measure was used. When data were
given graphically, we contacted authors seeking data; when this was
not available we estimated values by measurement from publica-
tions. Data were extracted onto a data extraction form by 2 reviewers
independently, and differences were resolved by discussion.
We also collected other relevant data including anesthetic used,
time of outcome measurement, and method of induction of ischemia,
as well as the individual component items of the quality checklist.

Analysis

For continuous variables (infarct volume, neurobehavioral score), we
calculated an overall weighted mean difference with a random effects
model. When a single control group served multiple treatment groups,
the size of the control group entered to the meta-analysis was adjusted
by division by the number of treatment groups served. Meta-analyses
were conducted stratified according to: drug dose; time of administra-
tion; study quality; components of study quality checklist; presence of
cotreatments; duration of ischemia; method of induction of ischemia;
anesthetic used; use of mechanical ventilation; species and gender of
animal used; outcome measure used; and interval to quantification of
outcome. The significance of differences between n groups was assessed
by partitioning heterogeneity and by using the

\[ \chi^2(n-1, df) \]

distribution with n−1
degrees of freedom (df). To allow for multiple comparisons we set our
significance level at P<0.001. Publication bias was assessed with a
funnel plot and with the Egger method.7

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Results

Electronic searching identified 19 publications describing the effect of tirilazad in focal cerebral ischemia; hand-searching did not identify any further relevant data. One of the identified studies reported infarct volume as median and interquartile range, but the author was unable to provide us with the raw data. This analysis is therefore based on 18 publications, 17 full articles, and 1 abstract (Table 1). Within these 18 studies, 34 comparisons were identified describing outcome in 544 animals. All 18 publications reported infarct volume, 8 also reported a neurobehavioral outcome (242 animals), and 1 reported infarct volume, neurobehavioral score, and mortality (24 animals).

Study Quality and Publication Bias

No study described a sample size calculation or contained a statement of potential conflict of interest, although 2 of the 18 publications were directly funded by and a further 2 studies describe some form of assistance from the manufacturers of tirilazad. The median quality score was 5 (interquartile range, 4 to 6), and whereas this compares favorably with similar reviews for other candidate neuroprotective drugs, this still represents an important potential source of bias (Table 2).

Efficacy

Tirilazad reduced infarct volume by 29.2% (95% confidence interval, 21.1% to 37.2%; 34 comparisons; Figure 1a), and improved neurobehavioral score by 48.1% (95% confidence interval, 29.3% to 66.9%; 16 comparisons; Figure 1b). There was substantial heterogeneity for both outcome measures (infarct volume, \( \chi^2 = 120, \text{df}=33, P < 10^{-12} \); neurobehavioral score, \( \chi^2 = 35.8, \text{df}=15, P = 0.002 \)). Only one study reported mortality data and was not analyzed further.

Data for infarct volume were used for prespecified stratified meta-analyses. There was a narrow window of therapeutic effect, with doses between 3 and 9.9 mg/kg giving maximum efficacy and doses only slightly lower (1 to 2.9 mg/kg) or higher (10 to 29 mg/kg) being only half as effective.
quality is associated with higher estimates of efficacy, and
quality checklist. We have previously shown that low study
evaluating concordance between animal and human studies

This review was conducted in the context of a project
examining concordance between animal and human studies

Lack of Concordance With Human Studies

This review was conducted in the context of a project
examining concordance between animal and human studies

for six interventions. Clinical trials in ischemic stroke showed
tirilazad increased death and disability. While one explana-
tion for this lack of concordance might indeed be that the
animal experiments were falsely positively as described,
comparison with tPA, a drug with efficacy in both animal and
human strokes, suggests 2 further plausible explanations.
First, the animal data suggest that tirilazad is effective over a
narrow dose range (3 to 10 mg/kg). Other aspects of study
design may confound this finding, and a single experiment of
adequate power would be required to confirm it; however,
these data are consistent with the narrow effective dose range
reported for another free radical scavenger, PEG-SOD. A
narrow dose range for tirilazad would contrast with tPA, for
which efficacy in animals was seen across a broad range of
doses (Sena E and Macleod M, unpublished observations,
2006). Clinical trials of tirilazad used a broad range of doses
(0.15 to 15 mg/kg), with no difference in outcome when
patients were dichotomized to those receiving more than or
less than 6 mg/kg/d; if the narrow effective dose range
suggested by our analysis also exists in humans, this might
explain why the clinical studies were negative.

Second, the interval between stroke onset and the initiation
of treatment was substantially longer in clinical studies
(median, ≈5 hours) than in the animal studies (median, 10
minutes). This contrasts with tPA, for which the median delay
to treatment in the animal studies, 90 minutes, is broadly
similar to the time window in which clinical efficacy has been
shown (within 180 minutes).

Because of concerns that findings in small laboratory
animals may not generalize to much larger human brains, the
STAIR criteria includes the demonstration of efficacy in
larger animals. The only gyrencephalic species in which
tirilazad was tested was in cats, in which no efficacy was
seen. Whereas this may reflect lack of efficacy in larger
animals, this was also the only experiment using female
animals; and partitioning heterogeneity by sex was of
borderline significance (P = 0.006). Establishing the source
of this lack of efficacy would require direct head-to-head
comparison.

Effects of Tirilazad in Animal Models

Tirilazad demonstrates substantial neuroprotective efficacy in
data from 18 publications in animal models of stroke.
However, this evidence must be interpreted with some
cautions. First, no study scored on >6 of 10 items on our
quality checklist. We have previously shown that low study
quality is associated with higher estimates of efficacy, and
of particular concern are the use of ketamine anesthesia,
unblinded assessment of outcome, and the small proportion of
studies using animals with a comorbidity. Some of the
efficacy of tirilazad may be caused by such bias. We did not
find evidence for significant publication confounded by such
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Discussion

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Lack of Concordance With Human Studies

This review was conducted in the context of a project
examining concordance between animal and human studies

(P < 0.001; Figure 2a). Although there was no significant
relationship between delay to treatment and efficacy it is
interesting to note that maximum efficacy was seen when
treatment was given before the onset of ischemia, with a trend
for efficacy to fall thereafter with time (Figure 2b). Although
the longest interval between stroke onset and initiation of
treatment was 6 hours, the median was only 10 minutes.
There was no relationship between study quality and treat-
ment effect. Efficacy was higher in temporary occlusion than
in either permanent or thrombotic occlusion models (χ² = 16.5, df = 2, P < 0.001; Figure 3a), and was lower in the
presence of comorbidity (4 comparisons undertaken among
spontaneously hypertensive rats; no other comorbidities were
tested) than in healthy animals (χ² = 26.6, df = 1, P < 0.001; Figure 3b). Tirilazad nonsignificantly increased infarct
volume in cats, whereas it improved outcome in both rats and
rabbits (χ² = 14.9, df = 2, P for difference between species
< 0.001; Figure 3c). There was no significant effect of the
anesthetic used; the use of cotreatments; single or multiple
dosages; the route of drug administration; the sex of animal
used; the method of quantification of infarct volume; or the
use of mechanical ventilation (not shown).

Figure 3. Effect of (a) duration of occlusion, (b) comorbidity, and (c) species on the estimate of efficacy. The shaded gray bar
represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% confidence intervals 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).
Robustness of These Conclusions

Although we prespecified our choice of stratification variables and set a very stringent significance level, some of our results may have been attributable to the play of chance; therefore, our observations should be interpreted with caution. This meta-analysis has other weaknesses. First, meta-analysis can only include available data, and publication bias may result in our analyses overestimating the efficacy of tirilazad. Furthermore, although we consider that our search strategy is likely to have ascertained most of the relevant publications, it has yet to be validated.

We elected to use weighted mean difference meta-analysis. We consider that standardized mean difference meta-analysis, although appropriate for clinical studies that individually have large numbers of participants, is less suited to animal studies in which the number of subjects is substantially lower. This is because the observed (sample) standard deviation used in standardization will be a poorer estimate of the population standard deviation when sample size is smaller. However, direct comparisons of the strengths and weaknesses of each statistical approach in this context have not yet been performed.

Third, our analysis is observational and should only be considered as hypothesis-generating. There were insufficient data to allow multiple linear regression analysis that could help disentangle the impact of different factors. However, by pooling such data from reviews of different neuroprotectors, it should be possible to identify those aspects of study quality for instance most closely associated with overstatement of efficacy. This work is underway.

This post-hoc approach allows potential sources of bias to be taken into account in the interpretation of published evidence for efficacy for candidate neuroprotective drugs. However, we believe that a better solution would be for the design, conduct, and reporting of animal studies to be refined to minimize the impact of these sources of bias.

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Disclosures

None.

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