

# Systematic Review and Stratified Meta-analysis of the Efficacy of Interleukin-1 Receptor Antagonist in Animal Models of Stroke

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*Background:* Interleukin (IL)-1 receptor antagonist (RA) is an anti-inflammatory protein used to treat arthritis that has also been identified as a candidate stroke drug. *Methods:* We conducted a systematic review and meta-analysis of reports of the efficacy of IL-1 RA in animal models of focal cerebral ischemia. *Results:* We identified 16 published sources and one unpublished source of data. IL-1 RA reduced infarct volume by 38.2% (95% confidence interval 31.2%-45.1%). Efficacy was higher with higher doses, earlier treatment, and central administration of drug. No studies used animals with hypertension or diabetes or tested efficacy beyond 3 hours. *Conclusions:* The animal data supporting IL-1 RA as a candidate drug for stroke are limited, and further experiments are required before proceeding to clinical trial. **Key Words:** Meta-analysis—systematic review—study quality bias—neuroprotection—publication bias.  
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The development of neuroprotective drugs for ischemic stroke has been characterized by success at the bench followed by failure at the bedside. More than 500 drugs have reported efficacy in animal models of stroke, but with the exception of tissue plasminogen activator, this efficacy has not translated to clinical trials.<sup>1</sup> This discrepancy might arise if the animal studies were falsely positive, the clinical trials were falsely negative, or the animal studies did not recapitulate human stroke with sufficient fidelity to be a useful guide to clinical efficacy. The development of candidate stroke drugs might, therefore, be improved by greater focus firstly on potential sources of bias in animal studies that might overstate efficacy and secondly on the design of clinical studies, so that these test drugs under circumstances similar to those where efficacy was seen in animal experiments.

Systematic review and meta-analysis of data from animal models of stroke is an emerging technique<sup>2</sup> that allows a description of the range of circumstances under which a drug shows efficacy and, hence, how the drug performs against, for example, the stroke treatment academic industry roundtable (STAIR) criteria<sup>3</sup>; a description of the quality of included studies and, hence, how the evidence performs on published checklists of study quality<sup>4</sup>; an assessment of the magnitude of any publication bias; and a precise overall estimate of drug efficacy in animal studies, of efficacy in subgroups of studies, and of the limits to efficacy.

Interleukin (IL)-1 receptor antagonist (RA) is an endogenously occurring 180 amino acid protein that is a competitive inhibitor at the IL-1 receptor. After ischemic brain injury there is a rapid up-regulation of the IL isoform IL-1 $\beta$  that is associated with neutrophil infiltration, neurotoxicity, and the promotion of apoptosis. IL-1 RA blocks many of these effects and, for instance, was reported to reduce cerebral edema in animal models of stroke.<sup>5,6</sup> IL-1 RA is well established as a treatment for rheumatoid arthritis, where it appears to be safe and well tolerated.<sup>7</sup> Taken together, these observations identify IL-1 RA as a candidate treatment for ischemic stroke. A single clinical trial showed that IL-1 RA was probably safe in acute stroke, but the study was not powered to detect efficacy.<sup>8</sup>

Here we report a systematic review and meta-analysis of the efficacy of IL-1 RA in animal models of stroke.

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Specifically, we set out to describe the range and quality of data, overall efficacy, determinants of efficacy (in particular drug dose, time of administration, and route of drug delivery), and areas of remaining uncertainty in the animal data where further animal research may be helpful, the better to define the therapeutic potential of IL-1 RA.

## Methods

A study protocol defining the methodology to be used and the stratified analyses to be performed was defined before any data collection and refined after external peer review (N. Rothwell, PhD, J Ross, PhD, personal communications 2006).

### *Search Strategy*

We searched PUBMED, EMBASE, and BIOSIS for [(interleukin 1 receptor antagonist) OR (IL-1 RA) OR (IL1 RA) OR (IL1-RA) OR (Anakinra)] AND [(stroke) OR (ischemia) OR (cerebrovascular) OR (middle cerebral artery) OR (MCA) OR (ACA) OR (anterior cerebral artery) OR (MCAO)] AND (Animals) NOT (coronary OR myocardial), and hand searched conference abstracts from the World Stroke Congress, European Stroke Conference (1992 onward), International Stroke Conference (2000 onward), International Society for Cerebral Blood Flow and Metabolism (1993 onward), Society for Neuroscience, Federation of European Neuroscience Societies (FENS), British Society for Immunology, and American Association of Immunologists. Two investigators (V. B. and M. R. M.) searched independently and screened potentially relevant abstracts, differences being resolved by discussion with a third investigator (E. S. S.).

### *Inclusion Criteria and Selection of End Points*

We included data describing outcome in whole live animal models of ischemic occlusive stroke of the middle cerebral or anterior cerebral arteries or their branches where animals were treated with IL-1 RA via any means of delivery (e.g., transgenic, viral, peripheral) and where outcome was compared with that in control animals receiving vehicle or no treatment. The primary end point was infarct area or volume; secondary end points were neurobehavioral score and death.

### *Data Extraction*

We extracted data for each comparison reported. Where a control group served more than one treatment group, the size of the control group used for the meta-analysis was adjusted accordingly. Where end points were measured serially the last measurement was recorded and where data were presented graphically authors were contacted seeking original data, failing which estimates were measured from the graph. We recorded the time of drug administration, cumulative drug dose in the first

24 hours of drug, route of drug delivery, type (permanent/temporary/thrombotic) and method of occlusion, time to outcome measurement, anesthetic used, whether or not animals were ventilated during surgery, method of infarct measurement, funding source, publication status, and the species and sex of animals used. Where full data were not available from publications or abstracts, these were requested directly from authors.

### *Range of Evidence*

We assessed the range of evidence against the STAIR criteria (3), that is: (1) evidence from two or more laboratories; (2) from two or more species; (3) from animals with comorbidities; (4) from male and female animals; (5) from both permanent and temporary models of ischemia; (6) testing at least two doses of drug; (7) with some doses given at least 1 hour after vessel occlusion; (8) testing using a feasible route of drug delivery; (9) of use of both histologic and neurobehavioral outcomes; (10) outcome measured at least 4 weeks after vessel occlusion; and (11) from species other than rodents.

### *Quality of Evidence*

We assessed the susceptibility to bias of each publication using the 10-item collaborative approach to meta-analysis and review of animal data in experimental studies (CAMARADES) study quality checklist<sup>4</sup> (peer-reviewed publication; control of temperature; randomization to treatment or control; blinded induction of ischemia; blinded assessment of outcome; avoidance of anesthetics with marked intrinsic neuroprotective properties; use of animals with comorbidities [e.g., hypertension or diabetes]; sample size calculation; statement of compliance with animal welfare requirements; and statement of possible conflicts of interest).

### *Analysis*

Data were analyzed as previously described.<sup>4</sup> Briefly, outcome measures were normalized to outcome in the control group and combined by weighted mean difference meta-analysis using DerSimonian and Laird random effects modeling. Prespecified subgroup analyses were carried out for drug dose; time of administration; mode of delivery; study quality; components of study quality checklist; presence of cotreatments; method of induction of ischemia; anesthetic used; use of mechanical ventilation; species and sex of animal used; outcome measure used; and interval to quantification of outcome. Publication bias was assessed with a funnel plot and with the method of Egger et al.<sup>9</sup> To allow for multiple testing we set a significance level of *P* less than .001.

## Results

In all, 88 publications (82 publications identified electronically, 6 by hand searching) were identified, of which

16 (15 full publications, one abstract)<sup>10-25</sup> met our inclusion criteria. Requests to authors for unpublished data provided a further 7 comparisons from one group (Clark S, PhD personal written communication 2006). Five studies reported outcome with adenoviral delivery of IL-1 RA, one reported outcome in transgenic animals over-expressing IL-1 RA, and 11 reported outcome with IL-1 RA administered as a drug (Fig 1). Characteristics of these 17 studies are shown in Table 1.

Only one publication reported neurologic score and only two studies reported mortality and so these end points were not considered further. This analysis is, therefore, based on data from 44 comparisons from 17 sources reporting infarct volume in a total of 784 animals.

Overall, IL-1 RA reduced infarct volume by 38.2% (95% confidence interval [CI] 31.2%-45.1%,  $P < .001$ ) with significant heterogeneity between studies ( $I^2$  heterogeneity statistic [percentage of variability between studies due to heterogeneity rather than chance] = 64.4%,  $P < .001$ ). Infarct volume was reduced by 38.6% (95% CI 31.0%-46.2%) in 37 comparisons (686 animals) where IL-1 RA was given, by 44.7% (95% CI 33.4%-56.0%) in 6 comparisons (88 animals) where IL-1 RA expression was increased using a viral vector, and there was a nonsignificant worsening of outcome (-4.2%, 95% CI -24.2% to 15.8%) in one study involving 10 animals where IL-1 RA expression was increased in transgenic animals. Individual comparisons, grouped according to the mode of IL-1 RA delivery, are shown in Fig 2. Because of uncertainty about the timing or effective dose achieved in transgenic and transfection studies, further analyses were restricted to data from 11 sources describing 37 comparisons where IL-1 RA was administered directly.

There was evidence for efficacy under a range of conditions, meeting 7 of the STAIR criteria. There were no data from animals other than rats or mice, from animals with comorbidities, or for outcome beyond 1 month. The median quality score was 4 (interquartile range 3-5) (Table 2). Randomization and allocation concealment were each reported in one of 11 sources, and the blinded assessment of outcome was reported in 3 of 11 sources; no study reported a sample size calculation or declared whether or not a potential conflict of interest existed. Although partitioning studies by quality score accounted for a significant proportion of the observed heterogeneity ( $P < .0001$ ) the nature of the relationship between quality score and effect size (Fig 3) was not clear. Funnel plotting suggested an excess of imprecise studies giving higher than anticipated estimates of efficacy and this was confirmed by Eggar regression ( $P < .001$ ; Funnel plot not shown).

Dose response relationships were analyzed separately according to whether IL-1 RA protein was delivered centrally (intracerebroventricular) or peripherally (intravenous, intranasal). A significant dose response relationship was only seen for central drug administration and, as expected, the systemic dose required to achieve protection was substantially higher than the intracerebroventricular dose. Furthermore, the maximum protection was greater with central compared with peripheral administration ( $P < .001$ ) (Fig 4, A).

Time of treatment ranged from 30 minutes before to 180 minutes after induction of ischemia. Neuroprotection was maximal when drug was administered before ischemia or in the first 60 minutes thereafter; there were limited data beyond 60 minutes, and the confidence limits of the

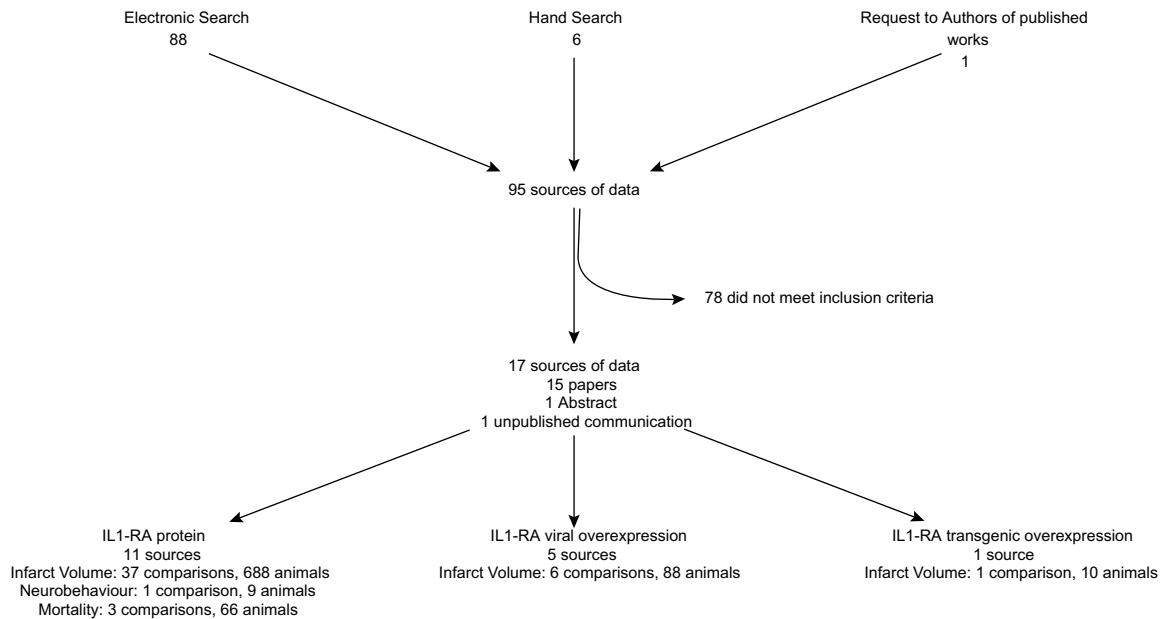


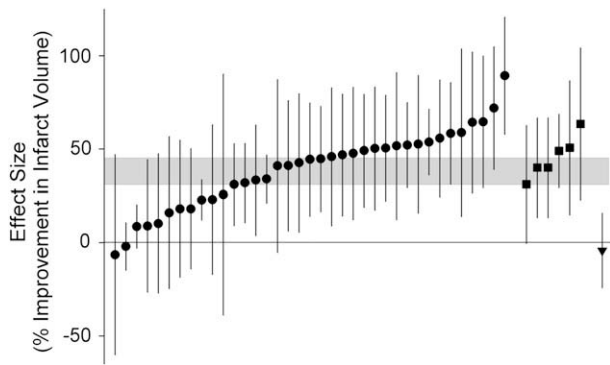
Figure 1. Results for search and selection of studies and experiments for inclusion.

**Table 1.** Study characteristics

| Study                         | Species | No. of animals | Dose              | Time of admin (min) | Anesthetic      | Type of ischemia occlusion model | Route of delivery | Outcome measure(s)        | Modality     |
|-------------------------------|---------|----------------|-------------------|---------------------|-----------------|----------------------------------|-------------------|---------------------------|--------------|
| Betz et al <sup>10</sup>      | Rat     | 12             | 9.1 ng/g          | -7200               | Isoflurane      | Permanent                        | icv               | Infarct volume            | Viral vector |
| Boutin et al <sup>11</sup>    | Mouse   | 23             | 5 µg              | -30                 | Halothane       | Temporary                        | icv               | Infarct volume            | Protein      |
| Clark*                        | Rat     | 167            | 10-29,200 µg      | 0-90                | Halothane       | Temporary                        | iv                | Infarct volume            | Protein      |
|                               |         |                |                   |                     |                 | Permanent                        | icv               | mortality                 |              |
| Clark et al <sup>25</sup>     | Rat     | 14             | 29,200 µg         | 0                   | Halothane       | Temporary                        | iv                | Infarct volume            | Protein      |
| Garcia et al <sup>12</sup>    | Rat     | 45             | 112,000 µg        | 0                   | Ketamine        | Permanent                        | iv                | Infarct volume            | Protein      |
|                               |         |                |                   |                     |                 |                                  |                   | neurobehavioral mortality |              |
| Le Feuvre et al <sup>13</sup> | Mouse   | 14             | 10 µg             | 0                   | Halothane       | Temporary                        | icv               | Infarct volume            | Protein      |
| Loddick et al <sup>14</sup>   | Rat     | 99             | 10-20 µg          | -30-0               | Halothane       | Permanent                        | icv               | Infarct volume            | Protein      |
| Mao et al <sup>15</sup>       | Mouse   | 16             | -                 | -7200               | Isoflurane      | Temporary                        | icv               | Infarct volume            | Viral vector |
| Mulcahy et al <sup>16</sup>   | Rat     | 84             | 20 µg             | 0-180               | Halothane       | Temporary                        | icv               | Infarct volume            | Protein      |
| Oprica et al <sup>17</sup>    | Mouse   | 10             | -                 | -                   | Chloral hydrate | Permanent                        |                   | Infarct volume            | Transgenic   |
| Relton et al <sup>19</sup>    | Rat     | 210            | 12,500-250,000 µg | 0                   | Halothane       | Permanent                        | iv                | Infarct volume            | Protein      |
| Relton et al <sup>18</sup>    | Rat     | 24             | 20 µg             | -30                 | Halothane       | Permanent                        | icv               | Infarct volume            | Protein      |
| Stroemer et al <sup>20</sup>  | Rat     | 72             | 5-7.5 µg          | 0                   | Halothane       | Permanent                        | icv               | Infarct volume            | Protein      |
| Touzani et al <sup>21</sup>   | Mouse   | 16             | 5 µg              | -30                 | Halothane       | Temporary                        | icv               | Infarct volume            | Protein      |
| Tsai et al <sup>22</sup>      | Rat     | 20             | 0.77 ng/g         | -                   | Chloral hydrate | Temporary                        | icv               | Infarct volume            | Viral vector |
| Yang et al <sup>23</sup>      | Mouse   | 28             | -                 | -7200               | Isoflurane      | Temporary                        | icv               | Infarct volume            | Viral vector |
| Yang et al <sup>24</sup>      | Mouse   | 12             | 2 ng/g            | -7200               | Isoflurane      | Temporary                        | icv               | Infarct volume            | Viral vector |

Abbreviations: admin, administration (in relation to onset of ischemia); icv, intracerebroventricular; in, intranasal; iv, intravenous.

\*Unpublished communication.



**Figure 2.** Individual comparison and grouped according to whether IL-1 RA was delivered as preformed protein (circles) or whether expression was increased using adenoviral transfection (squares) or transgenic approaches (triangle) and ranked according to effect on infarct volume. Shaded gray bar represents 95% confidence limits of global estimate of efficacy. Vertical error bars represent 95% CI for individual estimates.

estimate were wide and consistent with there being no effect (Fig 4, B).

Efficacy was significantly higher in temporary compared with permanent models of ischemia (44.0% v 34.8%,  $P < .001$ ) (Fig 5, A), in studies correcting observed infarct volume for the presence of edema (43.2% v 25.2%,  $P < .001$ ) (Fig 5, B), and in studies reporting the blinded assessment of outcome (45.6% v 34.0%,  $P < .001$ ) (Fig 5, C). Random allocation to experimental group and allocation concealment were each reported in the same single publication comprising 8 comparisons, and although reported efficacy was higher in studies without randomiza-

tion or allocation concealment these differences did not reach statistical significance (Fig 5, D).

There was no effect of the delay to assessment of outcome; frequency of drug dosing; anesthetic used; species (rat or mouse) or sex of animals used; whether published in full, in abstract, or available as an unpublished communication; or of route of drug delivery.

**Discussion**

IL-1 RA appears to have substantial neuroprotective efficacy in experimental stroke, with a global estimate of efficacy of a 38% reduction of infarct volume. However, this conclusion must be qualified because there was significant heterogeneity between studies; the range of conditions under which efficacy was tested was narrow; study quality was modest when scored against established checklists; and there was evidence consistent with a substantial publication bias.

*Range and Quality of Evidence*

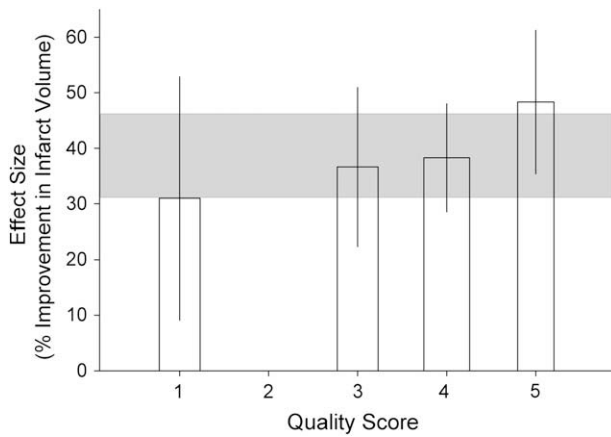
The range of evidence available was limited, and of particular concern is the lack of evidence at times of administration beyond 180 minutes, of testing in animals with hypertension or diabetes, and of testing in larger animals. Data for other neuroprotective drugs where such data are available suggest that efficacy may be substantially lower under these conditions.<sup>2,4</sup>

**Table 2.** Study quality items

| Author                        | Year | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | Quality score |
|-------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|---------------|
| Betz et al <sup>10</sup>      | 1995 | +   | +   |     |     |     | +   |     |     |     |      | 4             |
| Boutin et al <sup>11</sup>    | 2001 | +   | +   |     |     | +   | +   |     |     | +   |      | 5             |
| Clark et al <sup>25</sup>     | 2005 |     | +   |     |     |     |     |     |     |     |      | 1             |
| Clark*                        | 2005 |     | +   |     |     | +   | +   |     |     | +   |      | 4             |
| Garcia et al <sup>12</sup>    | 1995 | +   | +   |     |     | +   |     |     |     | +   |      | 4             |
| Le Feuvre et al <sup>13</sup> | 2003 | +   | +   |     |     |     | +   |     |     | +   |      | 4             |
| Loddick et al <sup>14</sup>   | 1996 | +   | +   |     |     |     | +   |     |     |     |      | 4             |
| Mao et al <sup>15</sup>       | 2000 | +   | +   |     |     |     | +   |     |     |     |      | 4             |
| Mulcahy et al <sup>16</sup>   | 2003 | +   | +   |     |     | +   | +   |     |     | +   |      | 5             |
| Oprica et al <sup>17</sup>    | 2004 | +   | +   |     | +   | +   | +   |     |     | +   |      | 6             |
| Relton et al <sup>18</sup>    | 1992 | +   | +   |     |     |     | +   |     |     |     |      | 3             |
| Relton et al <sup>19</sup>    | 1996 | +   |     | +   | +   |     | +   |     |     |     |      | 5             |
| Stroemer et al <sup>20</sup>  | 1997 | +   | +   |     |     |     | +   |     |     |     |      | 3             |
| Touzani et al <sup>21</sup>   | 2002 | +   | +   |     |     |     | +   |     |     | +   |      | 4             |
| Tsai et al <sup>22</sup>      | 2003 | +   | +   |     |     |     | +   |     |     |     |      | 3             |
| Yang et al <sup>23</sup>      | 1997 | +   | +   |     |     |     | +   |     |     | +   |      | 5             |
| Yang et al <sup>24</sup>      | 1999 | +   | +   |     |     |     | +   |     |     | +   |      | 5             |

(1) Peer-reviewed publication; (2) control of temperature; (3) randomization to treatment or control; (4) blinded induction of ischemia; (5) blinded assessment of outcome; (6) avoidance of anesthetic with marked intrinsic neuroprotective properties; (7) use of animals with comorbidities such as hypertension or diabetes; (8) sample size calculation; (9) statement of compliance with animal welfare requirements; and (10) statement of possible conflict of interest.

\*Unpublished communication.



**Figure 3.** Effect of study quality on estimate of efficacy. Shaded gray bar represents 95% confidence limits of global estimate. Vertical error bars represent 95% CI for individual estimates. Width of each bar reflects log of number of animals contributing to that comparison. Stratification by study quality accounts for significant proportion of heterogeneity observed between studies ( $P < .001$ ).

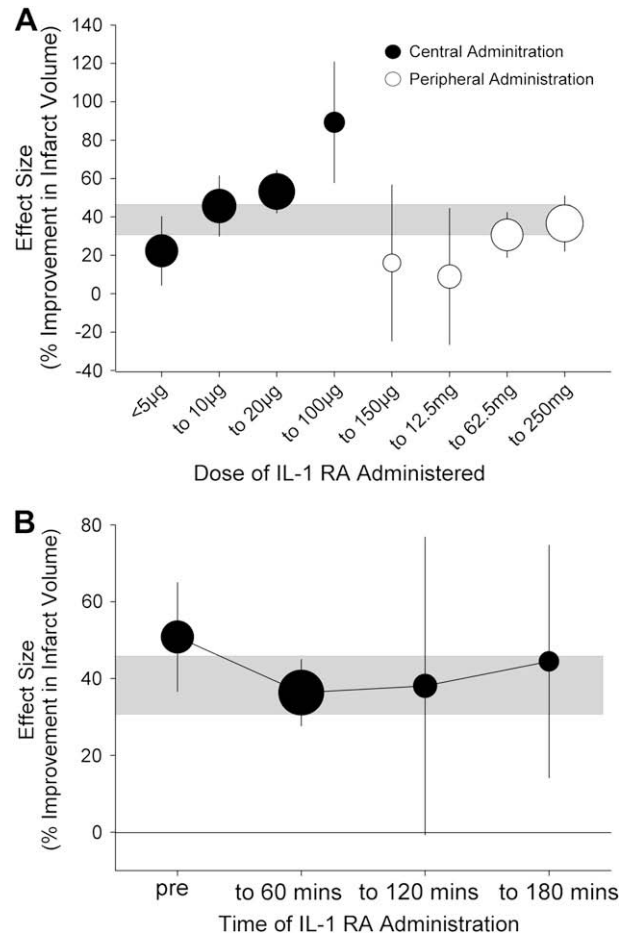
#### Efficacy in Prespecified Subgroups

We examined whether stratifying studies according to predetermined criteria explained some of the substantial heterogeneity observed between studies. There was a robust dose response effect and, as expected, IL-1 RA is more potent when delivered centrally. Partitioning of heterogeneity also suggested a significant increased efficacy in temporary compared with permanent occlusion models, an effect also described for other neuroprotective drugs and consistent with the view that neuroprotection is more difficult to achieve in permanent occlusion models.

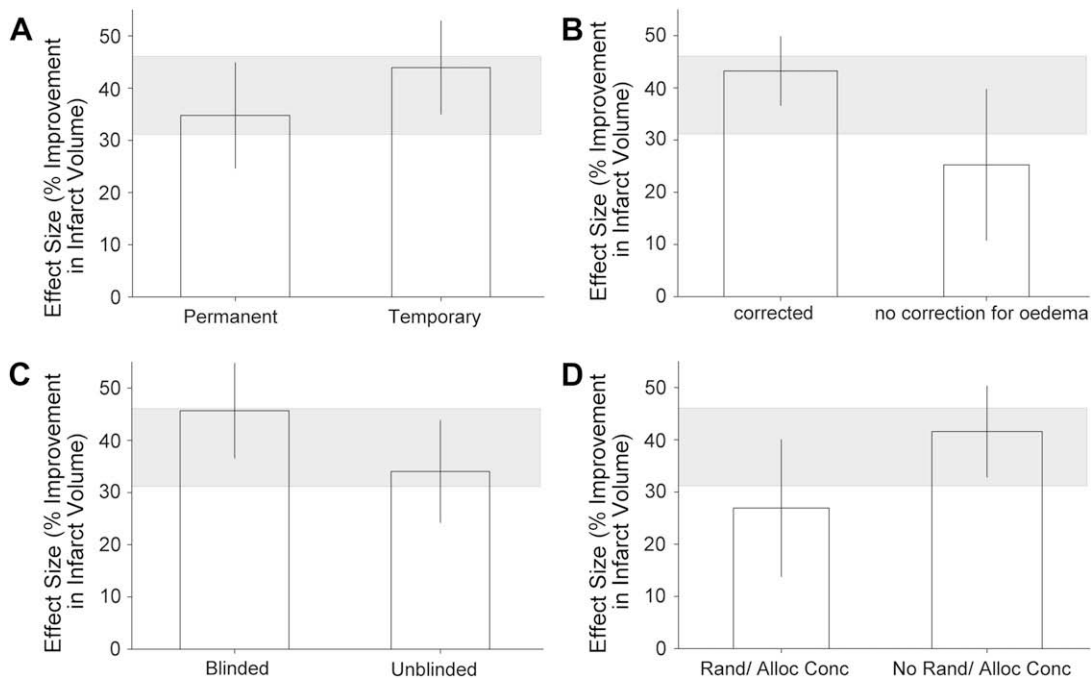
Perhaps more surprising was the impact of two factors that might be expected to reduce the estimate of efficacy, the blinded assessment of outcome and the correction of infarct volume for the presence of edema. In each case the reverse was in fact observed, with greater efficacy in higher quality studies. These observations illustrate some of the difficulties with such a univariate analysis; two of 3 studies reporting the blinded assessment of outcome used central rather than peripheral administration of drug, and the third used ketamine anesthesia, which is known to augment the protective effects of other candidate neuroprotective drugs. We have not shown whether these associations between aspects of study quality and improved outcome represent causal links or are a result of the simultaneous presence of other factors; there are insufficient data for IL-1 RA to allow for multivariate analysis.

Efficacy is highest when drug is present at or soon after the onset of ischemia, and although there is limited testing of efficacy beyond 30 minutes, the evidence that does exist suggests a decline in efficacy; there was no evidence for efficacy for treatment initiated more than 180 minutes after the start of ischemia. Hopkins and Roth-

well<sup>26</sup> have shown that IL-1 is rapidly up-regulated after the induction of ischemia, with messenger RNA levels increased within 15 minutes and protein levels increased within 60 minutes of stroke onset. It is not clear when IL-1 levels peak after cerebral ischemia or for how long they remain elevated, but in models of traumatic brain injury levels of IL-1 $\beta$  are reported to peak at around 8 hours postinjury and return to normal at around 16 hours.<sup>27</sup> It is not known whether the deleterious effects of IL1 are more pronounced in the early stages of exposure or spread evenly throughout the period of exposure, but the suggestion from such observations that the time window for the efficacy of IL-1 RA in stroke may extend beyond the first hours has yet to be confirmed in focal ischemia studies. Given the substantial difficulties in recruiting patients to clinical trials in these first few hours after stroke we suggest that the efficacy of peripherally administered IL-1 RA at delays of up to 6 hours after the onset of ischemia should be established before proceeding to clinical trial.



**Figure 4.** Effect of IL-1 RA dose (A) and delay to treatment (B) on estimate of efficacy. Shaded gray bar represents 95% confidence limits of global estimate. Vertical error bars represent 95% CI for individual estimates. Size of each point reflects log of number of animals contributing to that comparison. Stratification by dose, but not by time to treatment, accounts for significant proportion of heterogeneity observed between studies ( $P < .001$ ).



**Figure 5.** Effect of temporary or permanent occlusion ( $P < .001$ ) (A), correction of measured infarct volume for presence of edema ( $P < .001$ ) (B), blinding of assessment of outcome ( $P < .001$ ) (C), and randomization (rand) and allocation (alloc) concealment (conc) (not significant) (D). Shaded gray bar represents 95% confidence limits of global estimate. Vertical error bars represent 95% CI for individual estimates. Width of each vertical bar reflects log of number of animals contributing to that comparison.

#### Potential Problems with this Analysis

Although our stratifications were prespecified and a stringent significance level was chosen to allow for multiple testing, some of our results may be a result of chance and all data should, therefore, be interpreted with caution. This meta-analysis has other weaknesses. First, although we consider our search strategy likely to have ascertained most relevant publications, it has yet to be validated. Furthermore, we have only been able to include data that were published in some form, and it is likely that publication bias has led to an over-estimation of efficacy.

Second, we have elected to use normalized 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 where the number of subjects is substantially lower. This is because the observed (sample) SD used in standardization will be a poorer estimate of the population SD where sample size is smaller.

#### Implications for Translating Efficacy to Clinical Trial

Only one clinical trial of IL-1 RA in stroke was reported<sup>8</sup>; 25 patients with stroke were randomly allocated to either IL-1 RA or placebo within 6 hours of stroke and outcome assessed 3 months later. IL-1 RA appeared to be safe, but the trial was too small to allow conclusions about efficacy to be drawn.

Our analysis suggests that further data from high-quality animal experiments are required, particularly with regard to efficacy at later time points, in animals with comorbidities, and in animals other than rodents. Currently, the animal data do not support the clinical use of IL-1 RA at more than 60 minutes after stroke onset, and a requirement for treatment to be delivered within this time limit is likely severely to limit any clinical use.

Our analysis also shows that IL-1 RA is substantially more effective when delivered directly to the ventricular system than when it is given peripherally. It may be that increased systemic dosage is able to overcome this and achieve the levels of efficacy seen with central delivery, but this may come at the cost of systemic side effects, and again should be the subject of further animal experiments.

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