Stem cell-based therapy for experimental stroke: A systematic review and meta-analysis

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Stem cell therapy holds great promise in medicine, but clinical development should be based on a sound understanding of potential weaknesses in supporting experimental data. The aim of this article was to provide a systematic overview of evidence relating to the efficacy of stem cell-based therapies in animal models of stroke to foster the clinical application of stem cell-based therapies and to inform the design of large-scale clinical trials. We conducted a systematic search for reports of experiments using stem cells in animal models of cerebral ischaemia, and performed DerSimmonian and Laird random effects meta-analysis. We assessed the impact of study characteristics, of publication bias and of measures to reduce bias. We identified 6059 publications, 117 met our prespecified inclusion criteria. One hundred eighty-seven experiments using 2332 animals described changes in structural outcome and 192 experiments using 2704 animals described changes in functional outcome. Median study quality score was 4 (interquartile range 3 to 6) and less than half of studies reported randomization or blinded outcome assessment; only three studies reported a sample size calculation. Nonrandomized studies gave significantly higher estimates of improvement in structural outcome, and there was evidence of a significant publication bias. For structural outcome autologous (i.e. self-derived) stem cells were more effective than allogeneic (donor-derived) cells, but for functional outcome, the reverse was true. A significant dose–response relationship was observed only for structural outcome. For structural outcome, there was an absolute reduction in efficacy of 1·5% (–2·4 to –0·6) for each days delay to treatment; functional outcome was independent of the time of administration. While stem cells appear to be of some benefit in animal models of stroke the internal and external validity of this literature is potentially confounded by poor study quality and by publication bias. The clinical development of stem cell-based therapies, in stroke and elsewhere, should acknowledge these potential weaknesses in the supporting animal data.

Key words: meta-analysis, stem cells, stroke, systematic review, translation

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Introduction

The ability to repair or even recreate damaged or senescent functional systems, tissues or even whole organs would have profound effects on both the quality and duration of human life. Continuing advances in our understanding of stem cell biology and in our ability to derive stem cells and modulate their phenotype provides a platform for the next phase of therapeutic development, the demonstration of efficacy in animal models of human disease. In recent years there has been an explosion of articles in the scientific and the popular press suggesting that stem cell therapies may transform modern medicine, and the literature surrounding stroke is no exception.

While over 1000 drugs have been tested in laboratory studies, and over 400 have reported efficacy in animal models of stroke, therapies are still limited. Attempts to develop new treatments have been characterized by substantial reported efficacy in animal studies that has not translated to subsequent clinical trials. This is probably due to a combination of an overstatement of efficacy in animals (due to study quality and design limitations and to publication bias) and that clinical trials have not tested efficacy under circumstances where it was seen to be maximal in animals. Given the substantial

public interest in the potential use of stem cells a further source of bias, what Sackett called 'Hot Stuff Bias' (1), may also be present.

Systematic reviews have advantages over narrative reviews in fields such as stem cell research because they consider all available information rather than simply those publications carried in journals of high impact or those known to (and perhaps favored by) authors of narrative reviews. While a global estimate of efficacy for stem cells in stroke models is perhaps of little use, it is possible to determine the proportion of studies reporting measures to reduce bias (and therefore to infer the internal validity of a cohort of data), to identify whether publication bias may be a problem, and, using meta-analysis and meta-regression, to examine the impact of various aspects of study design on reported outcome (2,3).

Stem cells used to treat stroke can be considered as 'autologous' or 'allogeneic'. In the former, cells derived from an animal are extracted, may be manipulated, and are then returned to the same animal. In the latter, cells – embryonic or adult – derived from a different animal are administered to a recipient animal. Stem cells may be delivered either directly to the brain or given systemically in the expectation they will manifest tropism to brain and specifically to damaged tissues. The use of pharmacological strategies to mobilize endogenous stem cells is not considered here.

To foster the clinical application of stem cell-based therapies in stroke and to inform the design of large-scale clinical trials we need better to understand the optimal stem cell approach, the ways in which they might contribute to repair and recovery, and to understand any limitations of the existing data. Here, we report a systematic review and meta-analysis of published reports describing the use of stem cells in animal models of focal cerebral ischemia. We use normalized mean difference random effects meta-regression to assess the impact of various determinants on outcome and looked for evidence of publication bias. Our objectives were as follows:

- to summarize the evidence for the effectiveness of stem cell efficacy in animal models of stroke
- to ascertain the conditions of maximum efficacy; and
- to assess the internal and external validity of reported findings.

Methods

Identification of relevant studies

Electronic searching of four electronic databases in October 2009 (Pubmed, EMBASE, BioSIS, and ISI Web of Science) for (stem cell or stem or hematopoietic or mesenchymal) and (stroke or cerebrovascular or ischemia or middle cerebral artery or MCA or MCAO or ACA or ACAO or anterior cerebral artery), with the search limited to animal studies.

Inclusion and exclusion criteria: we included controlled studies that reported the efficacy of allogeneic or autologous stem cells in animal models of focal cerebral ischemia where the outcome was expressed as a change in structural (infarct size) or functional (neurobehavioral) outcome where we could determine the number of animals in each group, the mean effect size, and its variance. We excluded studies where interventions such as growth factors were used to mobilize endogenous stem cells.

Data extraction

We extracted details of experimental design from each publication. Study quality was assessed according to a published checklist (4) comprising: publication in a peer-reviewed journal and statements describing control of temperature, randomization to treatment group, allocation concealment, blinded assessment of outcome, avoidance of anesthetics with known marked intrinsic neuroprotective properties, use of animals with relevant comorbidities; sample size calculation, compliance with animal welfare regulations, and whether the authors declared any potential conflict of interest. For the calculation of an aggregate study quality score one point was attributed for each checklist item reported.

From each experiment, we extracted data for reported outcome. Where a publication reported more than one treatment group (for instance, the impact of different delays to treatment or number of stem cells transplanted) we considered these to be independent experiments and extracted data for each of these (correcting the weighting of these studies in meta-analysis to reflect the number of treatment groups served by each control group). Structural and functional outcomes were analyzed separately and so where both were reported we extracted both. Where different functional outcomes were reported in a single cohort of animals we combined these using fixed effects meta-analysis to give a summary estimate of functional outcome. Where outcomes (functional or magnetic resonance imaging-based estimation of structural outcome) were reported from the same cohort of animals at different times we extracted data for the last outcome reported.

Analysis

For each experiment, we calculated a normalized effect size as the percentage improvement in outcome in the treatment group and then used DerSimonian and Laird random effects weighted mean difference meta-analysis to calculate summary estimates of global effect size and of efficacy in prespecified subgroups; results are presented as the percentage improvement, with 95% confidence interval (CI), of outcome in the treatment group. We looked for publication bias using funnel plot, Egger regression, and trim and fill (5).

The analysis was stratified according to: (i) the approach to stem cell therapy (allogeneic or allogeneic, embryonic, or adult); (ii) biological factors (number of cells, time and route of administration, time of assessment of outcome); (iii) aspects of study design (anesthesia, species of animal, comor-

bidities, immunosuppression, model of ischemia); and (iv) elements of study quality. We assessed the extent to which stratification into subgroups explained difference between studies using meta-regression with a significance level of $P < 0{\cdot}002$ to allow for multiple comparisons.

Results

Characteristics of included studies

Electronic searching identified 6059 publications of which 117 met our prespecified inclusion criteria. Of these, 70 reported both structural and functional outcome, 11 reported structural outcome (infarct volume) alone, and 36 reported functional (neurobehavioral) outcome alone. Characteristics of included studies are given in Appendix 1(a).

The first report of the use of stem cells in focal ischemia was in 1998 when the Lund group investigated whether striatal implantation of conditionally immortalized rat neural stem cells engineered to produce nerve growth factor (NGF) improved outcome following middle cerebral artery occlusion. They found that NGF-secreting cells, but not unmodified neural stem cells, improved histological outcome, while there was no effect on a neurobehavioral test (6). The first report of experiments designed specifically to test the hypothesis that stem cells themselves might improve outcome was published in 1999 by Fukanaga and colleagues and described the use of fragments of E10·5 rat mesencephalic neural plate tissue, and while they were able to show survival of engrafted tissue and reduced escape latencies in the Morris Water Maze in a subset of animals in whom graft survival was seen, overall their results were disappointing (7). The following year, the Chopp group published two reports of the effect of donor bone marrow-derived cells, showing evidence again supporting an effect on functional rather than structural outcome (8,9).

Since that time, a further 113 publications have contributed to this field, testing efficacy across a range of conditions described in the Supporting Information Appendix S1(a). Twenty-three publications described the use of stem cells engineered to produce various factors including brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, and vascular endothelial growth factor. The vast majority of these used adenoviral transfection of rat or human bone marrow-derived stem cells (Appendix 1b).

Internal validity and publication bias

Median study quality score was 4 (interquartile range 3–6). Randomization was reported in 46% of experiments, allocation concealment in 19% and the blinded assessment of outcome in 42%. Study quality items reported by individual studies are given in Appendix 1c. For structural outcome, nonrandomized studies reported efficacy more than 10% higher than that seen in randomised studies [29·1% (24·3–33·9) vs. 18·7% (11·3–26·1); $P < 0{\cdot}05$]. There was no significant effect of allocation concealment or blinded assessment of outcome. For functional outcome, there was no significant effect of randomization, allocation concealment, or blinded assessment of outcome.

For structural outcome, modest funnel plot asymmetry was detected consistent with the presence of publication bias, and this was confirmed with Egger regression (Fig. 1c,d). Trim and fill identified one missing study (Fig. 1e). Although Egger regression found no significant publication bias for functional outcome (Fig. 1f,g), trim and fill suggested 52 missing studies and a reduction in effect size from 40·6% (37·1–44·0) to 26·7% (23·0–30·3; Fig. 1h).

Impact of various aspects of study design

In total, 187 experiments using 2332 animals reported that structural outcome was improved by 24·8% (95% CI 21·5–28·1; $P < 0{\cdot}001$; Fig. 1a), and 192 experiments using 2704 animals reported that functional outcome was improved by 40·6% (37·1–44·0; $P < 0{\cdot}001$; Fig. 1b).

Compared with autologous cells, allogeneic stem cells were less effective at improving structural outcome but more effective in improving functional outcome (Fig 2a,b). There was no difference between embryonic and adult allogeneic cells for either outcome.

A significant dose–response relationship was observed only for structural outcome, where there was also a significant decline in efficacy of 1·5% with increasing delays to treatment. No dose or delay to treatment effects were observed for functional outcome. We found no significant influence of the route of administration on either structural or functional outcome.

Stem cells may be purified or modified prior to treatment. We found that for functional outcomes efficacy was greater for differentiated, selected, or transfected cells compared with nonmodified cells. For structural outcome, efficacy was higher only for transfected and differentiated cells (Fig 2c–f). In a subset of experiments (Supporting Information Appendix S1b) where the efficacy of cells engineered to overexpress factors of interest was compared with 'wild type' stem cells, there were additional improvements in both structural [16·8% (8·4–25·3; $P < 0{\cdot}001$] and functional [31·3% (22·3–40·3; $P < 0{\cdot}001$] outcomes, although, no one strategy had clear superiority.

Only one publication used genetically immunodeficient animals (10), but some used ciclosporin to induce immunosuppression; findings from these experiments may be confounded by the reported neuroprotective effects of ciclosporin; however, interestingly, while functional outcome was improved, there was no effect on structural outcome (Fig 2h–g). Experiments using cotreatments modeling rehabilitation did not report greater efficacy. Experiments using ketamine anesthesia reported significantly higher efficacy for both structural and functional outcomes, but the impact of mechanical versus spontaneous ventilation was not clear Fig 2i–l).

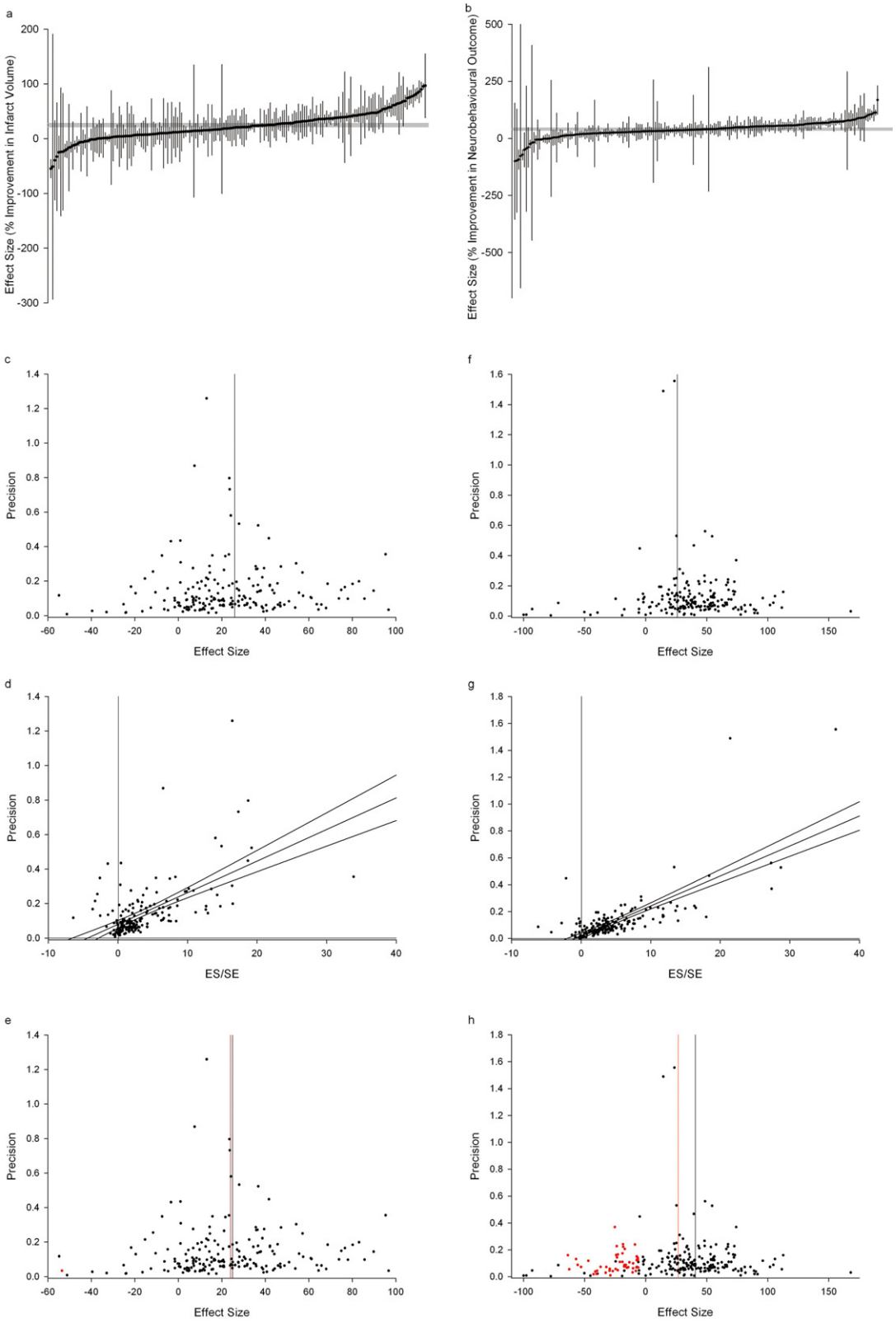

Fig. 1 Individual comparisons ranked according to their effect on (a) infarct volume and (b) neurobehavioral outcome. The shaded gray bar represents the 95% confidence limits of the global estimate. The vertical error bars represent the 95% confidence intervals for the individual estimates. Assessment of publication bias shown with funnel plots (c and d), Egger regression (e and f), and funnel plots showing the data in black and the additional missing studies imputed by trim and fill in red from (e and h) for studies reporting effect of stem cells on infarct volume or neurobehavioral outcome. The 95% confidence intervals of the Egger regression line do not include the origin, suggesting the presence of a significant publication bias. The red vertical line indicates the adjusted global estimate in the absence of publication bias.

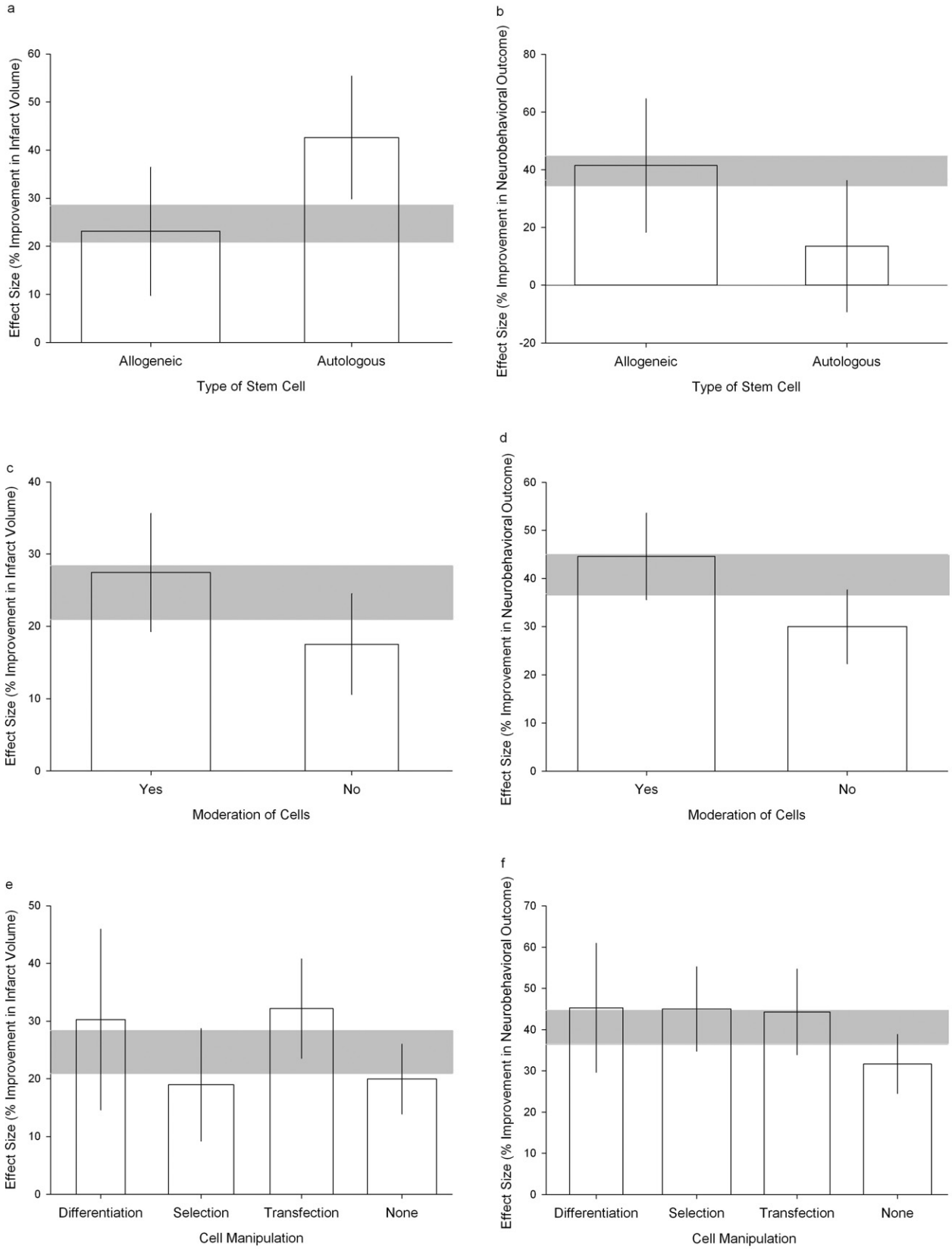

Fig. 2 The effect of type of stem cells on the estimate of improvement in (a) infarct volume and (b) neurobehavioral outcome. The effect of moderation of stem cells on the estimate of improvement in (c) infarct volume and (d) neurobehavioral outcome and the type of cell manipulation on (e) infarct volume and (f) neurobehavioral outcome.

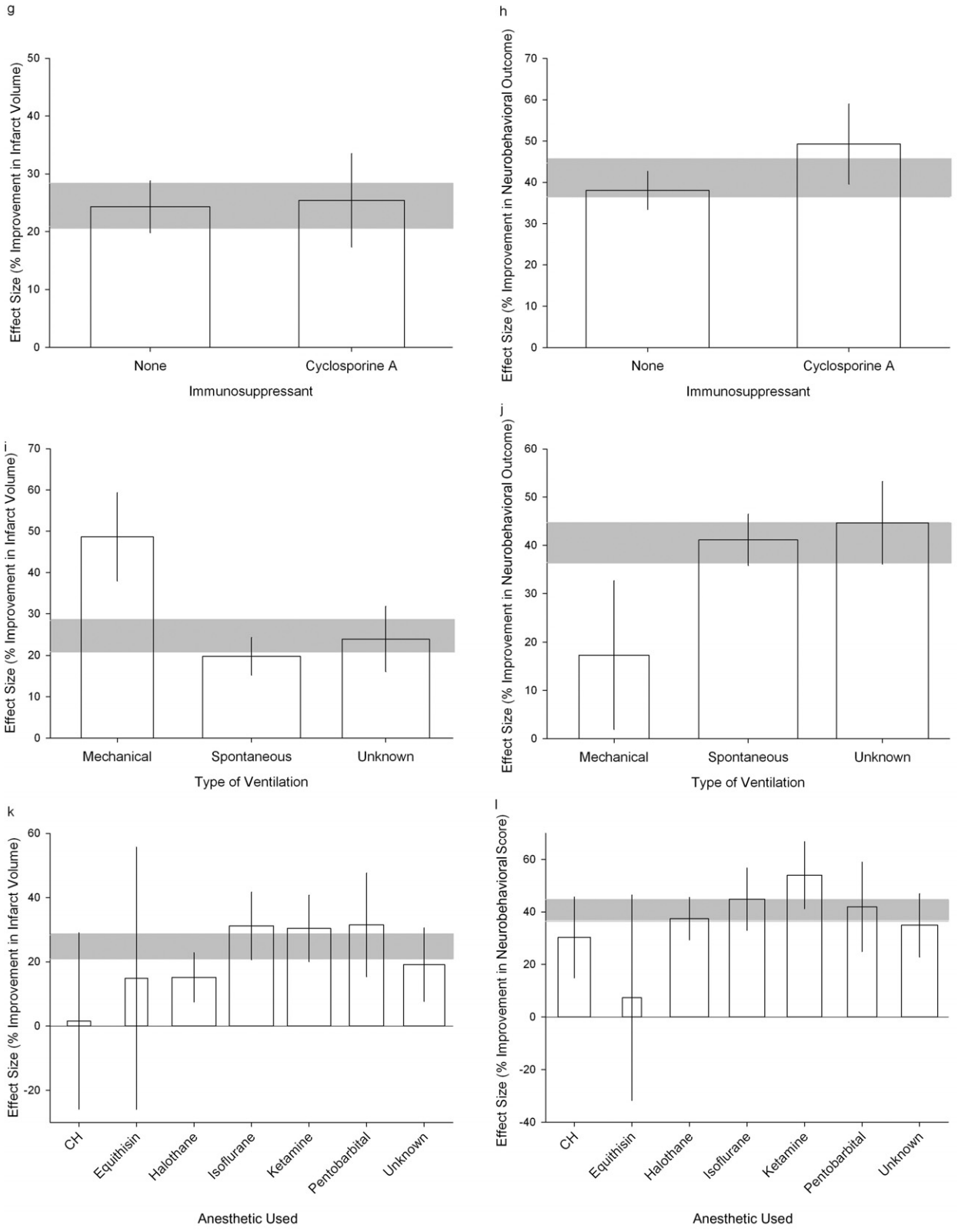

Fig. 2 *(Continued)* The impact of the use of immunosupression on the estimate of improvement in (g) infarct volume and (h) neurobehavioral outcome, the type of ventilation during anesthesia on the estimate of improvement in (i) infarct volume and (j) neurobehavioral outcome, and the impact on the anesthetic used on the estimate of improvement in (k) infarct volume and (l) neurobehavioral outcome. 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 column reflects the log of the number of animals contributing to that comparison. Each outcome accounts for a significant proportion of the heterogeneity observed between studies ($P < 0{\cdot}05$).

Discussion

Here we have summarized data from 119 publications identified through systematic review, and our conclusions are therefore likely to be less biased and more robust than those from narrative reviews. In brief, our findings suggest that for functional outcome, efficacy is highest with allogeneic cells, which have been selected, differentiated, or transfected; where immunosuppression is used, at least in the medium term; and where there are cotreatments (such as the ketamine). The delay to treatment and the route and number of stem cells used appear to be less important. While these findings do provide some guidance for the design of clinical trials, there is a low prevalence of measures to improve internal validity and a substantial risk of publication bias (5).

Our study has further limitations beyond the weaknesses deriving from the limited internal validity of included data and the likelihood that many data remain unpublished. Our approach is observational rather than experimental, and so we are only able to report associations rather than causation. While our search strategy was designed to be exhaustive, it is possible that some published studies were missed; nonetheless, our study is likely to have captured the majority of reports in this field, and therefore represents the most complete review to date of the use of stem cells in experimental stroke. Because of these weaknesses, hypotheses arising from this work need testing in appropriately designed adequately powered head-to-head experiments.

Notwithstanding these important caveats, our analysis provides support for some hypotheses regarding the biology of stem cell-based therapies. Specifically, our observation that while early treatment is important for efficacy against structural outcomes, there is no such time dependence for functional outcome supports the hypothesis that the beneficial effect of stem cells combines an early neuroprotective effect and a late effect on neuroregeneration, neuroplasticity, and/or angiogenesis. The further improvement of functional but not structural outcome with immunosuppression lends weight to the hypothesis that the sustained effects of stem cells require survival of stem cells at least in the medium term (11). However, the duration of immunosuppressive treatment needed, and whether long-term survival of transplanted cells is required, is not clear. This is important because if efficacy does not require long-term survival and integration of transplanted cells then these might be engineered firstly to have a limited lifespan (obviating the risks of tumourigenesis) and secondly to have secretory properties which enhance efficacy.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1a. Study characteristics report

Appendix S1b. Studies reporting the use of stem engineered to secrete factors

Appendix S1c. Study quality report

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