GRANULOCYTE- COLONY STIMULATING FACTOR IN EXPERIMENTAL STROKE AND ITS EFFECTS ON INFARCT SIZE AND FUNCTIONAL OUTCOME: A SYSTEMATIC REVIEW

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ABSTRACT

Background

Granulocyte-colony stimulating factor (G-CSF) shows promise as a treatment for stroke. This systematic review assesses G-CSF in experimental ischaemic stroke.

Methods

Relevant studies were identified with searches of Medline, Embase and PubMed. Data were extracted on stroke lesion size, neurological outcome and quality, and analysed using Cochrane Review Manager using random effects models; results are expressed as standardised mean difference (SMD) and odds ratio (OR).

Results

Data were included from 19 publications incorporating 666 animals. G-CSF reduced lesion size significantly in transient (SMD -1.63, $p<0.00001$) but not permanent (SMD -1.56, $p=0.11$) focal models of ischaemia. Lesion size was reduced at all doses and with treatment commenced within 4 hours of transient ischaemia. Neurological deficit (SMD -1.37, $p=0.0004$) and limb placement (SMD -1.88, $p=0.003$) improved with G-CSF; however, locomotor activity ($\geq$4 weeks post ischaemia) was not (SMD 0.76, $p=0.35$). Death (OR 0.27, $p<0.0001$) was reduced with G-CSF. Median study quality was 4 (range 0-7/8); Egger's test suggested significant publication bias ($p=0.001$).

Conclusions

G-CSF significantly reduced lesion size in transient but not permanent models of ischaemic stroke. Motor impairment and death were also reduced. Further studies assessing dose-response, administration time, length of ischaemia and long-term functional recovery are needed.

INTRODUCTION

Stroke has enormous consequences both for the individual and society. Finding an effective treatment for this burden is proving challenging and protection of the neurovascular unit (del Zoppo, 2006) might be achieved through enhancing reperfusion, modifying neuronal activity, and augmenting neurorepair. Of these, reperfusion is effective with alteplase (Wardlaw et al., 2002) whilst neuroprotection has not been shown to be effective to date (Bath et al., 2001; Lees and Muir, 2002; Shuaib et al., 2007). One neuroprotectant showing promise is recombinant granulocyte colony stimulating factor (G-CSF). Its pharmacological and side effect profile is well known since G-CSF is already licensed for use in other indications in humans.

G-CSF is a 207 amino acid glycoprotein cloned more than 20 years ago (Nagata et al., 1986). Its recombinant form is usually administered to patients with neutropenia to reduce the risk of sepsis, or to volunteers willing to donate haematopoietic stem cells (mobilised by G-CSF) for allogenic or autologous infusion. Endogenous G-CSF is produced by numerous cell types including monocytes (Vellenga et al., 1988) (the most abundant source), fibroblasts (Zucali et al., 1986), mesothelial and endothelial cells (Zsebo et al., 1988). G-CSF and its receptor are expressed in the penumbral region of ischaemic stroke (Schneider et al., 2005) and recent studies have highlighted its neuroprotectant properties as a possible therapy for cerebrovascular disease. G-CSF also stimulates the release of stem cells from the bone marrow and it could, therefore, also promote neurorepair (Sprigg and Bath, 2007).

In light of ongoing human clinical trials assessing G-CSF in stroke, the purpose of this systematic review was to review systematically the effects of G-CSF in

experimental stroke and, in particular, its effect on infarct size, motor impairment and death.

MATERIALS AND METHODS

Experimental (non-human) studies assessing the effects of G-CSF in ischaemic models of stroke (any species, age, sex and model) were sought with searches of Medline, Pubmed and Embase; search keywords included: 'stroke', 'cerebrovascular', 'thrombosis', 'brain', 'cerebral', 'cerebellum', 'middle cerebral artery', 'ischaemia', 'embolism' and 'G-CSF'. Searches were limited to animal studies. The reference lists of included articles and review articles were searched, and abstracts used to select relevant articles. Pre-specified exclusion criteria were used to aid selection and prevent bias and studies were included if the following were met: (i) a focal ischaemic stroke model, not global; (ii) treatment was in the acute/subacute phase i.e. <7 days, not chronic; (iii) no other potential neuroprotectants administered with G-CSF (i.e. confounded); (iii) measures on infarct size or functional outcome were performed; (iv) there was a control group; and (v) data was from an original article, not a letter or review article. Decisions on study inclusion and exclusion were made by TE and PB.

Data Extraction

Summary data on total infarct size, measured as volume or area (mm^3, percentage of normal brain, or mm^2) were extracted from all eligible papers up to June 2009. If given, infarct volume from total brain, cortex and subcortex were obtained separately. Volumes corrected for oedema were chosen in preference to uncorrected data. Similarly, information on vital status, weight (grams), Rotarod test (time spent on Rotarod expressed in seconds or percentage compared to baseline), motor impairment (low scores indicate a better outcome), foot fault (number of errors and percentage of total errors made), limb placement test (neurological scores) and locomotion (vertical movement and rearing) were also collected. If published studies used multiple groups (e.g. to assess dose-response

relationships) then the number of animals in the control group was divided by the number of comparison groups. When numerical values were not available and contacted authors were unable to provide necessary information, published graphs were enlarged from the publication and the size of axes and position of data points estimated using *Grab* (version 1.3.1) on an Apple Mac. Methodological quality was assessed using methods previously described (Gibson et al., 2006; Willmot et al., 2005) based on an 8-point STAIR criteria (STAIR 2009) with one point given for evidence of each of the following: presence of randomisation, monitoring of temperature (not just maintenance), assessment of effect by G-CSF dose, assessment of effect by time between stroke onset and treatment, masked outcome measurement, assessment of outcome at days 1–3, assessment of outcome at days 7–30, and assessment of outcome other than just lesion size. Two authors (TE and CG) independently assessed methodological quality and data extraction.

Analysis

Data were grouped before analysis by: (i) model type (permanent or transient ischaemia); (ii) species; (iii) time to treatment with G-CSF; and (iv) total dose of G-CSF. Data from each of these groups were analysed as forest plots using the Cochrane Review Manager software (version 4.2.10), as used in previous animal meta-analyses (Gibson et al., 2006; Willmot et al., 2005). Since heterogeneity was expected between study protocols (different species, stroke models, dose, time), random effect models were used. The results of continuous/ordinal data are expressed as Standardised Mean Difference (SMD), with 95% confidence intervals (CI), which allows data measured on different scales and in different species to be merged. The results of binary data are expressed as odds ratios (OR) with 95% CI. Studies were weighted by sample size and statistical significance was set at $p<0.05$. Publication bias was assessed by visually examining Begg's funnel plot

(standard error of SMD against the SMD); asymmetry in the plot was formally assessed using Egger's test (Egger et al., 1997).

RESULTS

Design of Studies

The initial search for studies identified 220 relevant publications (figure 1). Once pre-specified exclusion criteria were applied, a total of 19 publications were chosen for analysis (table 1); these came from 12 laboratories in 8 countries (China, France, Germany, Japan, South Korea, Taiwan, UK and USA). Only one negative study was identified in which G-CSF had a detrimental effect on behavioural function, caused brain atrophy and exaggerated the inflammatory response in the infarcted area (Taguchi et al., 2007). Studies excluded (table 2) were those administering G-CSF in combination with other agents such as stem cell factor (SCF) and those assessing chronic (i.e. >7 days post stroke) and global (transient bilateral common carotid artery occlusion) ischaemia. Of note, no functional improvement was seen with G-CSF in the studies of chronic (Zhao et al., 2007a) and global (Matchett et al., 2007) ischaemia; studies co-administering SCF reduced infarct volume in female mice (permanent ischaemia) by 50% (Toth et al., 2008) and improved functional recovery in acute and subacute phases (Kawada et al., 2006).

Of the 19 included publications, 14 studied rats, and 5 studied mice; hypertensive (1 of 18) and diabetic (1 of 18) rats were investigated but no studies in primates were identified. Some publications included more than one experimental condition, giving 44 studies in total (table 1). Studies included transient models of ischaemia (n=29) with vessel occlusion time ranging between 45 and 180 minutes. Permanent models were used in 12 studies; and a photothrombotic model, which is less likely to negatively affect survival, was used in 3 studies, each of which only assessed functional outcome. All photothrombotic models used direct illumination of the cortex after sensitisation with the dye rose bengal to produce a focal infarct. G-CSF

was given via various routes (subcutaneously n=30, intravenously n=12, and intra-peritoneum n=4) and at various dose regimens (total dose range 50 to 3000 mcg/kg) and time from onset of ischaemia (range from -96 hours to 1 week with 68% of animals having ischaemia for $\leq$2 hours). Infarct size was measured either histologically (staining with triphenyltetrazolium chloride [TTC], toluidine blue, haematoxylin and eosin, or cresyl violet) or with MRI evaluation (T2 weighted images).

Infarct Size

26 studies from 19 publications (table 1) assessed the effects of GCSF on lesion size in a total of 412 animals (318 rats, 94 mice). All studies measuring infarct volume had protocols which required that G-CSF be administered within 24 hours. 22 of 26 studies measured these effects following transient ischaemia (table 3, figure 2). The presence of publication bias is suggested by a positive Egger's test of asymmetry ($p<0.001$, figure 3). Overall, G-CSF significantly reduced lesion size in transient ischaemia (SMD -1.63, 95% CI -2.14 to -1.11, $p<0.00001$). In the 4 studies using a permanent model (56 animals), G-CSF did not significantly reduce lesion size (SMD -1.53, 95% CI -3.42 to 0.36, $p=0.11$). Significant reductions in lesion size were seen in both rats and mice in transient (figure 2) but not permanent ischaemia. When only including the 21 studies that reported lesion volume (in mm^3) in transient ischaemia, the weighted mean difference was -62.32 (95% CI -79.6 to -45.1); equivalent to a SMD of -1.59 (95% CI -2.12 to -1.06).

Motor Impairment

G-CSF significantly reduced neurological deficit (SMD -1.37, 95% CI -2.13 to -0.61, $p=0.0004$) in 11 studies (Komine-Kobayashi et al., 2006; Sehara et al., 2007a; Sevimli et al., 2009; Solaroglu et al., 2009; Taguchi et al., 2007; Yanqing et al., 2006). Impairment was measured at various time points post stroke (1, 2, 3, 7, 14,

21 and 35 days); beneficial effects of G-CSF were seen at all times but there was no correlation between reduction in impairment and time to measurement (r_s= 0.37, p=0.47). G-CSF increased time that animals stayed on a Rotarod (Gibson et al., 2005a; Lee et al., 2005; Schneider et al., 2006; Sevimli et al., 2009) (table 3) at 1 and 5 weeks post ischaemia. Similarly, improvements were seen in limb function as assessed by limb placement tests at 1 and 4 weeks post stroke. This was not reflected, however, in locomotor activity (at 4 and 5 weeks), as assessed by vertical movements / rearing, where there was no difference between G-CSF and control (SMD 0.76, 95% CI -0.98 to 2.51, p=0.35) (Shyu et al., 2004b; Taguchi et al., 2007).

Survival

Data on vital status was available in 6 studies (Gibson et al., 2005a; Schabitz et al., 2003; Schneider et al., 2005; Sevimli et al., 2009; Six et al., 2003; Yanqing et al., 2006); G-CSF reduced the odds of dying almost 4-fold (OR 0.27, 95% CI 0.14 to 0.51, p<0.0001).

Total G-CSF dose

G-CSF administration varied considerably between studies: in studies using higher doses, regimens divided the G-CSF dose over a number of days (table 1). In an indirect comparison assessing total administered dose in transient ischaemia, a significant reduction in lesion size occurred at all doses (dose range 50 to 3000 mcg/kg) (figure 4). None of the doses used in permanent ischaemia (50 to 350 mcg/kg) had a significant effect on infarct size. No studies compared lesion size with 2 or more doses of G-CSF but one study displayed impaired behavioural function with increasing doses of G-CSF (0.5 to 250 mcg/kg) (Taguchi et al., 2007).

Timing of treatment

G-CSF was administered pre-ischaemia in one study (Sevimli et al., 2009) and only one study compared time to treatment (Lee et al., 2005). Significant reductions in lesion size in transient models of ischaemia were seen with treatment started within 4 hours post-ischaemia; trends to efficacy were also seen with commencement at 5 and 24 hours post-onset of ischaemia (data not shown).

Study Quality

The median study quality score was 4 (out of 8) with range 0 to 7. The majority of included studies were randomised (Gibson et al., 2005a; Gibson et al., 2005b; Han et al., 2008; Komine-Kobayashi et al., 2006; Lan et al., 2008; Lee et al., 2005; Schabitz et al., 2003; Schneider et al., 2005; Schneider et al., 2006; Sehara et al., 2007a; Sehara et al., 2007c; Sevimli et al., 2009; Solaroglu et al., 2006; Solaroglu et al., 2009; Yanqing et al., 2006; Zhao et al., 2007c) and used blinded outcome assessments (Gibson et al., 2005a; Gibson et al., 2005b; Komine-Kobayashi et al., 2006; Lan et al., 2008; Lee et al., 2005; Schabitz et al., 2003; Schneider et al., 2005; Schneider et al., 2006; Sehara et al., 2007a; Sevimli et al., 2009; Shyu et al., 2004a; Solaroglu et al., 2006; Solaroglu et al., 2009; Taguchi et al., 2007; Yanqing et al., 2006; Zhao et al., 2007c) and although each study varied in G-CSF dose regimen, only one study specifically addressed the optimal time window of drug administration (Lee et al., 2005). One study assessed dose response (Taguchi et al. 2007). After adjusting for the number of animals in each study, the effect of G-CSF on lesion size was not related with study quality (Spearman's rank correlation, r_s=-0.16, p=0.7).

DISCUSSION

This systematic review finds that G-CSF reduces lesion size in transient ischaemia within 4 hours of administration, motor impairment (neurological severity and limb function) and death in experimental stroke. No significant effects were seen in permanent ischaemic models or in long term locomotor activity. Of note, the identified studies were biased towards those which might be expected to be positive, i.e. most studies assessed transient (32 experiments) rather than permanent (12 experiments) ischaemia, and short rather than long ischaemia.

The mechanisms of action of these potential effects are under investigation and are probably multi-factorial. First, neuroprotective activity may be secondary to suppression of oedema formation (Gibson et al., 2005b), reduction of inflammation (Gibson et al., 2005b; Lee et al., 2005; Sehara et al., 2007a) (although G-CSF has also been reported to exacerbate the inflammatory response within the peri-infarct area (Taguchi et al., 2007)), and anti-apoptotic effects (with reduced cell death in the ischaemic penumbra) (Komine-Kobayashi et al., 2006; Schneider et al., 2005; Solaroglu et al., 2006). Second, CD34+ haematopoietic stem cells (which are mobilised by G-CSF) are able to migrate to the site of injury when administered either intracerebrally or intravenously (Jendelova et al., 2005; Sykova and Jendelova, 2005). Transplanted human bone marrow cells have been shown to generate neurons and astrocytes (Crain et al., 2005) but the ability of migratory stem cells to help restore functional and structural recovery post stroke has been questioned; in one study, bone marrow derived cells spontaneously fused, in vivo, with recipient Purkinje neurons in the cerebllum with no evidence of transdifferentiation (Alvarez-Dolado et al., 2003). Neurogenesis (Schneider et al., 2005; Sehara et al., 2007c; Shyu et al., 2004a) needs to be sufficient and angiogenesis (Lee et al., 2005; Sehara et al., 2007c; Shyu et al., 2004a) is also

necessary. In rats, G-CSF is able to cross the blood brain barrier when administered exogenously (Schneider et al., 2005) and both G-CSF and its receptor are widely expressed from neurons in the in the CNS (Schneider et al., 2005). In addition, a recent post mortem series highlighted G-CSF receptor upregulation in human ischaemic stroke (Hasselblatt et al., 2007). Though these findings are encouraging, species differences in the expression profile of G-CSF should be considered which could alter the effect it has on infarct volume reduction and functional recovery.

The optimal time of administration of G-CSF relative to stroke onset (0-4 hours) supports a role of neuroprotective mechanisms but whether neuroreparative actions occur is far less clear since only trends to efficacy were seen between 5 and 24 hours. However, these data were limited and neurorepair may need stimulation after 24 hours when inflammatory responses have declined (England et al., 2009). Nevertheless, further studies are required to determine the extent of the therapeutic window. Moreover, the most appropriate G-CSF dose and administration regimen still needs to be established. A positive correlation was seen between total G-CSF dose and lesion size reduction but the true dose-response relationship requires confirmation in a study designed to answer this.

Furthermore, the infarct type (transient or permanent ischaemia) requires consideration. Our analysis confirms efficacy of G-CSF in rodents with transient ischaemia within 4 hours of administration but a closer analogy to treating a large proportion of human stroke would be a permanent ischaemic model treated beyond 4 hours – a combination without evidence in this review. However, the data are limited and the smaller number of animals tested in the permanent ischaemic model may account for the lack of significance. Human stroke is also increasingly treated with thrombolytic agents and no articles were identified (except for one abstract (Kollmar et al., 2007)) assessing G-CSF in combination with tissue

plasminogen activator (t-PA). This is especially important for potential neuroprotective agents which have to be administered early. Other stroke models could also influence lesion volume and recovery, for example, the presence of co-morbidities such as diabetes, hypertension, hypercholesterolaemia, female species and increasing age. Ideally all should be tested for their confounding effects (Fisher et al., 2009).

However, the potential beneficial effects observed here could be artifactual in view of the presence of potential publication bias, detected for the effect of G-CSF on lesion size caused by transient ischaemic models. This suggests that neutral or negative studies were not published, or at least not identified despite using a comprehensive search strategy. It is possible that these studies have been published in a journal not included in MEDLINE or EMBASE, or that investigators deliberately did not publish neutral/negative data, or that such studies are not attractive to journal reviewers and Editors.

We assessed study quality on the basis of methodological recommendations derived from the STAIR consortium (STAIR, 1999) and found no relationship between study quality and effect on lesion size. STAIR standards were developed by an expert panel to address why so many clinical trials of neuroprotectants have failed, and we have previously used this scale in other meta-analyses of experimental regimes for stroke (Gibson et al., 2006; Willmot et al., 2005). Although the majority of studies in this review had written evidence of randomisation (16/19 publications) and blinded outcome assessment (16/19), important sources of bias if unused (Crossley et al., 2008; Macleod et al., 2009), other key methodological criteria were missing, including assessment of dose-response (1/19) and time-response (2 of 19). It is, of course, possible that these criteria were not reported rather than not being performed. Furthermore, the majority of studies reporting randomisation and

blinded outcome assessment was significantly higher than in other stroke reviews (Sena et al., 2007) and may explain why no study quality effect was observed here.

Meta-analysis methodology has a number of limitations and several caveats need to be identified for this systematic review. First, its findings depend on the success of identifying all relevant studies; the non-inclusion of some studies, perhaps due to non-publication ('publication bias') means that the estimated treatment effects may be an over- or an under-estimate. Second, the results depend on study quality (so assignment and observer bias are minimised). Third, study design determines what data are available for each included study; e.g. Rotarod and limb placement assessments largely came from one study (Lee et al., 2005) thereby restricting interpretation. Fourth, differences in methodology and study quality limit interpretation and introduce heterogeneity in findings, a problem that is addressed, in part, by using random effects models; e.g. analysing using standardised mean differences allows for comparison of infarct size whether measured by volume, area or percentage. Last, the selection process of suitable publications has the potential to introduce its own bias.

Of note, an earlier systematic review was published whilst the current one was being analysed. This former review observed a 0.8% reduction in infarct volume per 1 μg/kg increase in G-CSF dose (Minnerup et al., 2008). However, it included fewer articles (13 publications), excluded studies appropriate for analysis (Hermann and Kilic, 2008; Sehara et al., 2007c; Taguchi et al., 2007) and found no evidence of publication bias.

In summary, G-CSF appears to have neuroprotective qualities, although the results are limited in nature and potentially biased. Nonetheless, G-CSF offers a potential multimodal therapy for ischaemic stroke and it is possible that meaningful

reductions in infarct volume and improvements in functional recovery are translated into human trials. However, further experimental studies are required, in particular, assessing permanent models of ischaemia, length of ischaemia, other species (such as primates), both sexes, and animals with other co-morbidities and of increasing age (i.e. mimicking patients with stroke) (Fisher et al., 2009). Studies also need to address optimal dose and route regimens, and elucidation of time responses. Acquiring this information is key since clinical trials assessing the safety of G-CSF in human stroke are already underway (Bath and Sprigg, 2006; Shyu et al., 2006; Sprigg et al., 2006).

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TITLES AND LEGENDS TO FIGURES

Figure 1

Summary of trial identification process

Figure 2.

Effect of G-CSF on lesion volume in transient middle cerebral artery occlusion (N, number of animals; SMD, standardised mean difference; 95% CI, 95% confidence interval; SD, standard deviation; MCAo, middle cerebral artery occlusion)

Figure 3.

Begg's funnel plot showing the relationship between lesion volume (standardised mean difference, SMD) and standard error of SMD. Smaller neutral or negative studies (i.e. where lesion volume was not altered by G-CSF or increased with it) are missing suggesting the presence of publication bias (Egger's test $p<0.001$).

Figure 4.

Effect of total G-CSF dose (logged) on lesion volume in models of transient ischaemia. Data expressed as standardised mean difference (SMD) and 95% confidence intervals. Significant reductions in lesion volume: * $p<0.05$; ** $p<0.001$, # $p<0.0001$, ## $p<0.00001$

Table 1. Included Studies

Study	Parameters assessed [time of assessment]	GCSF dose (µg/kg)	Time to Treatment (hours)	Species	Model	Route	STAIR score
Gibson 2005a Experiment 1	Infarct volume (mm^3) [48 hours]	50	1	Adult male CL57 BL/6 mice	T 60mins	s.c.	6
Experiment 2	Rotarod, foot fault [daily for 7 days], Morris water maze [days 15 to 20]						
Gibson 2005b	Infarct volume (mm^3) [48 hours], survival	50	0	Adult male CL57 BL/6 mice	P	s.c.	4
Han 2008 Experiments 1-3	Infarct volume (mm^3) [4, 16 and 24 hours]	60	0.5	Male Wistar rats	T 60mins	i.v.	3
Kobayashi 2006 Experiments 1-2	Infarct volume (mm^3), neurological deficit (0-3 scale) [24 and 72 hours]	50	0.5	Adult male CL57 BL/6 mice	T 60mins	i.v.	4
Lan 2008 Experiment 1-3	Infarct volume (mm^3), NSS (0-18 scale) [days 7, 14 and 21]	50 for 7, 14 and 21 days	0	Male Sprague Dawley diabetic rats	P	s.c.	4
Lee 2005 Experiment 1	Infarct volume (mm^3) [day 1]	50 for 3 days	2	Male Sprague Dawley rats	T 90mins	i.p.	7
Experiments 2-4	Rotarod (% of baseline), MLPT (0-7 scale) [1 week before and weekly for 5 weeks post ischaemia]		2, 24, 96 and 168				
Schneider 2005 Experiment 1	Infarct volume (mm^3), survival	60	2	Male Wistar rats	T 90mins	i.v.	5
Experiment 2	Infarct volume (mm^3), Rotarod (seconds and AUC), NSS* (0-16 scale) [72 hours]	50	1		T 180mins		
Experiment 3	Rotarod (seconds and AUC), NSS* (0-16 scale) [weekly, 2 to 6 weeks post ischaemia]	15 for 5 days	1		Photo-thrombotic		
Schneider 2006 Experiment 1	Infarct volume (mm^3) [24 hours]	60	4	Male Wistar rats	T 90mins	i.v.	6
Experiments 2-3	Rotarod (seconds and AUC) [weekly, 1 to 6 weeks post ischaemia]	10 for 10 days	24 and 72		Photo-thrombotic		
Schabitz 2003	Infarct volume (mm^3) [24 hours], survival	60	0.5	Male Wistar rats	T 90mins	i.v.	4
Sehara 2007a Experiment 1	Infarct volume (mm^3) [72 hours]	50	1.5	Adult male Wistar rats	T 90mins	s.c.	4
Experiment 2	Neurological deficit (0-3 scale) [24 and 72 hours]						
Sehara 2007b	Infarct area (mm^2) [day 7]	50	1.5	Adult male Wistar rats	T 90mins	s.c.	2

Sevimli 2009 Experiment 1 Experiment 2 Experiment 3	Infarct volume (mm^3) [48 hours] Neurological deficit (0-5 scale) [24 and 48 hours] Rotarod (seconds), survival	250 bd for 6 days	-96	G-CSF deficient female C57BL/6 mice	T 45mins	s.c.	5
Shyu 2004 Experiment 1 Experiment 2	Infarct volume (mm^3) [day 7], Body swing test (% recovery) and locomotor activity (vertical movement) [days 1 to 28]	50 for 5 days	24	Adult Sprague Dawley male rats	T 90mins	s.c.	4
Six 2002	Infarct volume (mm^3) [4 days], survival	50	24	Male CL57 BL/6 Mice	T 60mins	s.c.	0
Solaroglu 2006 Experiment 1 Experiment 2 Experiment 3	Infarct volume (mm^3) [24 hours] Infarct volume (mm^3) [72 hours] Neurological deficit [24, 48 and 72 hours]	50 50 for 2 days 50 for 2 days	1.5	Adult male Sprague Dawley rats	T 90mins	s.c.	5
Solaroglu 2009 Experiment 1 Experiment 2	Infarct volume (mm^3) [24 hours] Neurological score (scale 3-18) [24 hours]	50	1.5	Adult male Sprague Dawley rats	T 90 mins	s.c.	5
Taguchi 2007 Experiment 1 Experiments 2-3	Infarct area (mm^2) [72 hours] Locomotor activiity (rearing) and neurological deficit [35 days]	50 for 3 days 0.5, 5, 50 or 250 for 3 days	24	CB-17 mice	P	s.c.	6
Yanqing 2006 Experiments 1-3	Infarct volume (mm^3), survival, NSS (0-18 scale) [7, 14 and 21 days]	10 for 5 days	5	Male Sprague Dawley rats	T 60mins	s.c.	4
Zhao 2007b Experiment 1 Experiments 2-3	Infarct volume (%) [12 weeks] Foot fault, limb placement test [1, 4, 7 and 10 weeks]	50 for 7 days	3	Male hypertensive rats	P	s.c.	4

T, transient ischaemia; P, permanent ischaemia; s.c., subcutaneous; i.v., intravenous; i.p., intraperitoneal; NSS, neurological severity score; AUC, area under the curve; MLPT, modified limb placement test

Table 2. Excluded studies

Study	Reason for exclusion and comments.
(Toth et al., 2008)	G-CSF given in combination with SCF in a permanent MCAo mouse model. Treatment induced infarct volume reduction and enhanced angiogenesis.
(Morita et al., 2007)	Assessed inflammatory marker expression in mice with permanent ischaemia treated with a combination of G-CSF and SCF in acute (1 to 10 days) and subacute (11 to 20 days) phases. No assessment on functional recovery or infarct volume
(Zhao et al., 2007a)	Treatment given to rats with chronic ischaemia (3.5 months post stroke). No benefit seen in functional outcome in rats given G-CSF alone. SCF alone and in combination with G-CSF improved functional outcome.
(Yata et al., 2007) (Kim et al., 2008)	Rat models of neonatal hypoxia. No assessments of infarct volume or functional recovery. Treatment with G-CSF improved quantitative brain weight (Yata et al., 2007) and inhibited apoptosis (Kim et al., 2008).
(Kawada et al., 2006)	G-CSF given in combination with SCF acutely and subacutely. Permanent MCAo model used. Reduction in infarct volume and enhanced functional recovery.
(Matchett et al., 2007)	Effect of G-CSF on global cerebral ischaemia. No long-term (2 weeks) protection seen in neurobehavioural studies.
(Zhao et al., 2007b)	Observed that G-CSF and SCF pass through an intact blood-brain barrier in intact rats. No stroke induced.
(Chen et al., 2005)	Article in Chinese and unable to acquire to translate.
(Willing et al., 2003)	Transplanted peripheral blood progenitor cells (mobilised by G-CSF) and human umbilical cord-blood derived stem cells in rats 24 hours after permanent MCAo. Compared to control, a reduced stroke-induced hyperactivity was observed in the transplanted animals.
(Hokari et al., 2009)	Bone marrow stromal cells (BMSC), pre-treated with G-CSF, transplanted into mouse stroke brain, enhanced motor function earlier than mice treated with 'non-treated' BMSC.
(Muller et al., 2009)	Compared neurotransmitter profile in rats subjected to photothrombotic ischaemia treated with either G-CSF or brain derived neurotrophic factor (BDNF). No infarct volumes or functional outcome measures.
(Sehara et al., 2007b)	In a rat model of transient MCAo, G-CSF enhanced cell proliferation in the dentate gyrus. No measures of infarct volume or functional outcome.

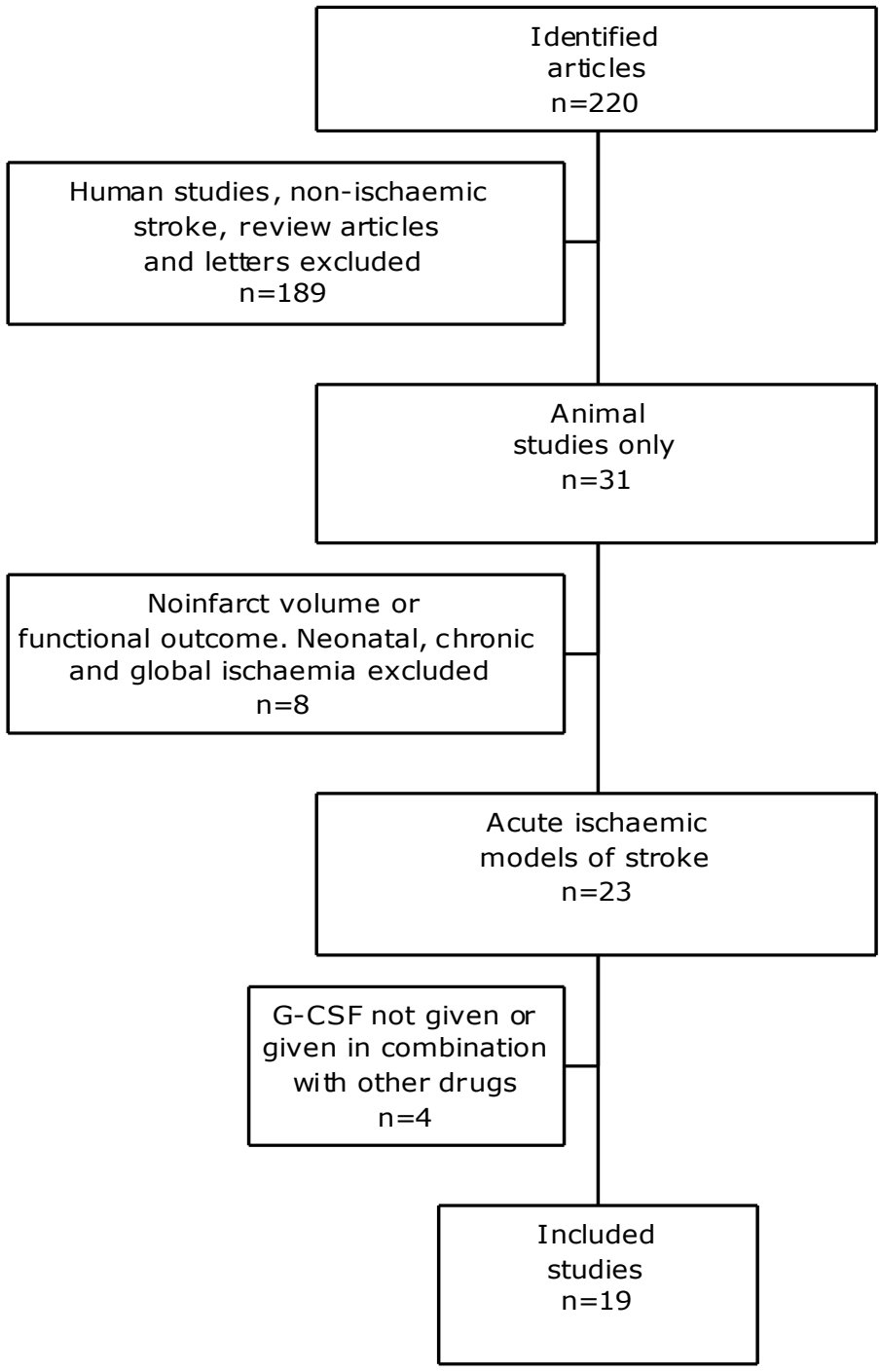
SCF Stem Cell Factor; MCAo middle cerebral artery occlusion

Table 3. Effect of G-CSF on lesion volume, functional outcome and survival
(SMD, standardised mean difference; OR, odds ratio; 95% CI, 95% confidence interval; IS, ischaemic stroke)

	N° of Studies	N° of Animals	SMD 95% CI	p-value
Lesion Volume				
Transient IS	22	356	-1.63 (-2.14, -1.11)	< 0.00001
Permanent IS	4	56	-1.53 (-3.42, 0.36)	0.11
Mice	7	94	-1.61 (-2.81, -0.40)	0.009
Rats	19	318	-1.58 (-2.12, -1.04)	< 0.00001
Motor Impairment				
Neurological deficit	11	108	-1.37 (-2.13, -0.61)	0.0004
Rotarod (≤1 week post IS)	5	87	1.11 (0.16, 2.06)	0.02
Rotarod (5 weeks post IS)	6	115	3.24 (1.63, 4.85)	< 0.0001
Foot fault (1 week post IS)	2	32	-0.45 (-2.10, 1.19)	0.59
Limb placement (1 week post IS)	5	95	-0.97 (-1.91, -0.04)	0.04
Limb placement (4 weeks post IS)	5	95	-2.17 (-3.61, -0.72)	0.003
Locomotor activity (≥4 weeks post IS)	5	54	1.75 (-1.89, 5.38)	0.35
	N° of Studies	**N° of Animals**	**OR 95% CI**	**p-value**
Survival				
Transient ischaemia	6	230	0.27 (0.14, 0.451)	< 0.0001

Figure 1

Summary of trial identification process



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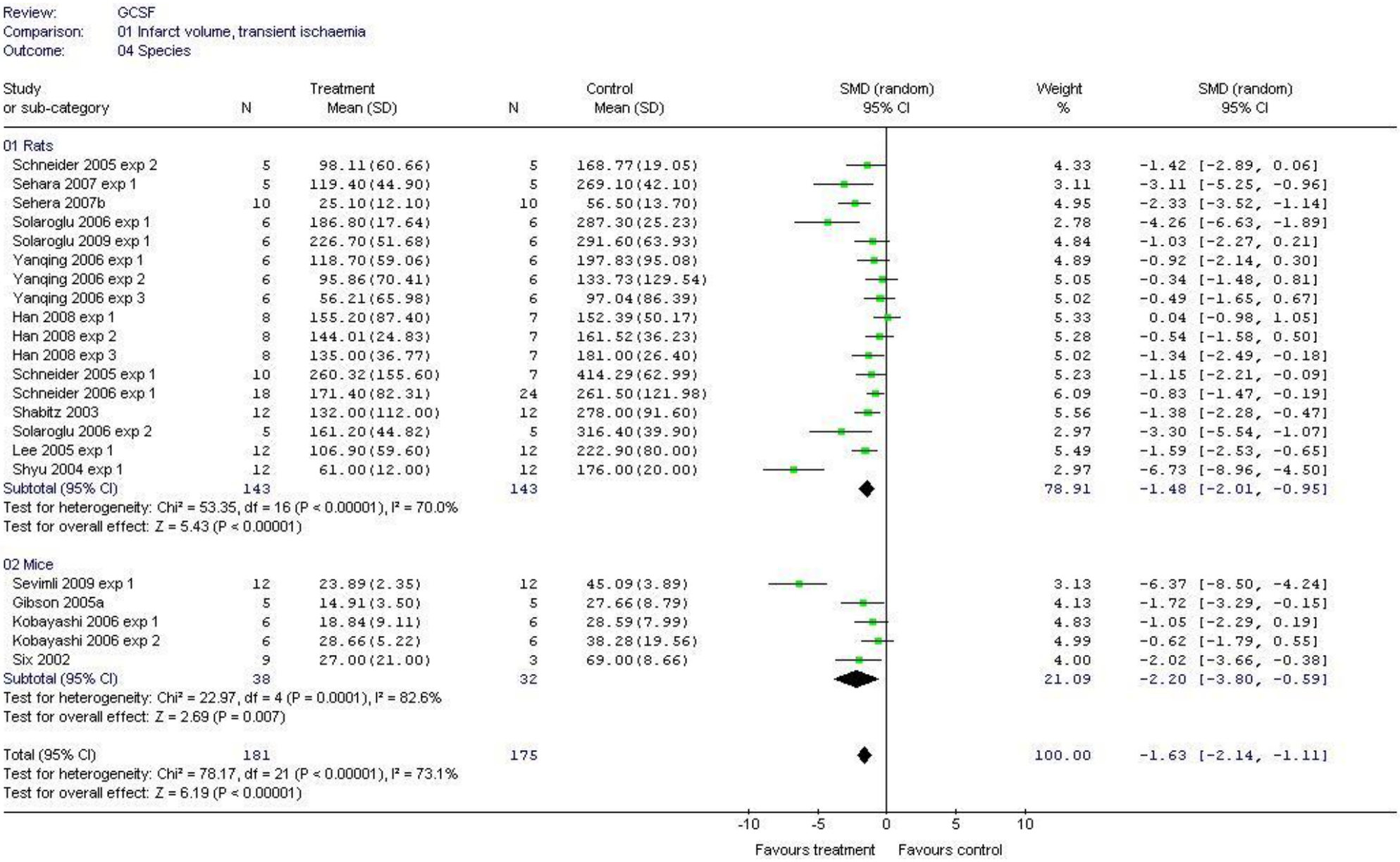
Effect of G-CSF on lesion volume in transient middle cerebral artery occlusion (N, number of animals; SMD, standardised mean difference; 95% CI, 95% confidence interval; SD, standard deviation; MCAo, middle cerebral artery occlusion)



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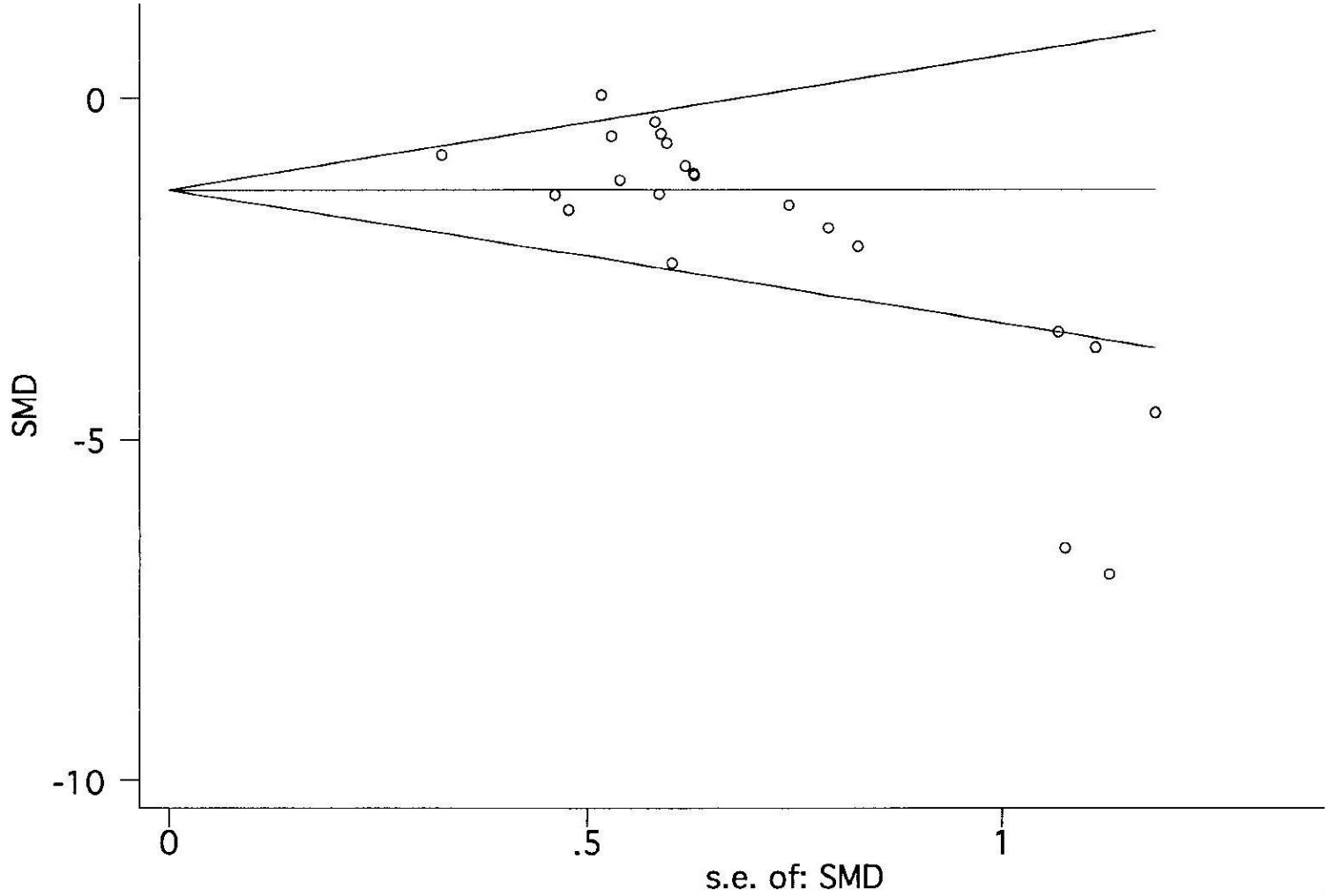

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