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Mesenchymal stem cells improve locomotor recovery in traumatic spinal cord injury: Systematic review with meta-analyses of rat models

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ABSTRACT

Traumatic spinal cord injury (SCI) is a devastating event with huge personal and societal costs. A limited number of treatments exist to ameliorate the progressive secondary damage that rapidly follows the primary mechanical impact. Mesenchymal stem or stromal cells (MSCs) have anti-inflammatory and neuroprotective effects and may thus reduce secondary damage after administration. We performed a systematic review with quantitative syntheses to assess the evidence of MSCs versus controls for locomotor recovery in rat models of traumatic SCI, and identified 83 eligible controlled studies comprising a total of 1,568 rats. Between-study heterogeneity was large. Fifty-three studies (64%) were reported as randomised, but only four reported adequate methodologies for randomisation. Forty-eight studies (58%) reported the use of a blinded outcome assessment. A random-effects meta-analysis yielded a difference in behavioural Basso–Beattie–Bresnahan (BBB) locomotor score means of 3.9 (95% confidence interval [CI] 3.2 to 4.7; $P < 0.001$) in favour of MSCs. Trial sequential analysis confirmed the findings of the meta-analyses with the upper monitoring boundary for benefit being crossed by the cumulative Z-curve before reaching the diversity-adjusted required information size. Only time from intervention to last follow-up remained statistically significant after adjustment using multivariate random-effects meta-regression modelling. Lack of other demonstrable explanatory variables could be due to insufficient meta-analytic study power. MSCs would seem to demonstrate a substantial beneficial effect on locomotor recovery in a widely-used animal model of traumatic SCI. However, the animal results should be interpreted with caution concerning the internal and external validity of the studies in relation to the design of future clinical trials.

Introduction

Spinal cord injury

Traumatic spinal cord injury (SCI) is a catastrophic and disabling event that results in severe motor, sensory, and autonomic dysfunction. The costs of SCI are enormous for the affected individual with a significant impact on quality of life and life expectancy (Boakye et al., 2012; Devivo, 2012; Geyh et al., 2013; Middleton et al., 2012). Almost 50% of all traumatic SCIs result in complete loss of function below the level of injury and the cost of medical care for the first year for a patient with high tetraplegia is estimated at $800,000 and for a patient with paraplegia at $300,000. Accordingly, if a person sustains a SCI at the age of 25, lifetime medical costs would surpass $3.3 million in the case of high tetraplegia and $1.1 million for paraplegia. Worldwide, 2.5 million people are affected with SCI, and every year more than 130,000 people sustain a traumatic SCI (Adams and Cavanagh, 2004). In addition, as many are estimated to contract a non-traumatic spinal cord lesion although the available data in many areas of the world are lacking (New et al., in press; Noonan et al., 2012).

In traumatic SCI, the primary damage refers to the mechanical impact that within seconds to minutes leads to a disrupted blood–brain-barrier, haemorrhages, disrupted axons, and broken neural-cell body membranes. A broad spectrum of progressive secondary damage soon follows (minutes to weeks), consisting of ischaemia, oedema, biochemical changes, and inflammatory cell responses that substantially aggravate the primary injury in rostro-caudal direction, thus affecting subsequent neural repair and regeneration (Hausmann, 2003; Oyinbo, 2011). Detrimental biochemical changes include excessive calcium influx-mediated neuron and glial apoptosis, glutamate excitotoxicity, lipid peroxidation, and nitrous oxide excess. Main inflammatory effector cells

Abbreviations: α, risk of type I error; β, risk of type II error; BBB, Basso–Beattie–Bresnahan; CI, confidence interval; CSPGs, chondroitin sulphate proteoglycans; D2, diversity; I2, inconsistency measure; IFNγ, interferon gamma; lacZ, betagalactosidase; M1, classically activated macrophage/microglia; M2, alternatively activated macrophage/microglia; MAG, myelin-associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein; MSC, mesenchymal stem cell; Nogo-A, neurite outgrowth inhibitor A; SCI, spinal cord injury; SD, standard deviation; SE, standard error; TSA, trial sequential analysis.

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comprise waves of activated neutrophils and classically activated (M1) blood borne macrophages and tissue-resident microglia (Beck et al., 2010; Fleming et al., 2006) which are associated with the rapid increase in neurotoxic matrix metalloproteinases, reactive oxygen species, and pro-inflammatory cytokines (Fleming et al., 2006; Nguyen et al., 2007; Noble et al., 2002).

Later, in the chronic phase, the spinal cord is characterised by continuing white-matter demyelinisation, atrophy, Wallerian degeneration, and central cavity formation in previously injured areas. Eventually, a developing glial scar is characterised by reactive astrocytes, activated resident microglia, invading macrophages, and potent neurite growth-inhibiting substances such as chondroitin sulphate proteoglycans (CSPGs), myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), and neurite outgrowth inhibitor (Nogo)-A (Bregman et al., 1995; Filbin, 2003; Freund et al., 2006).

In a contusion lesion, which is the most common morphological type of injury, the amount of primary macerated nervous tissue is relatively low. Instead, progressive secondary damage is more predominant, which in time may encompass several spinal segments above and below the original impact, thus extending the functional impairment. Especially the post-traumatic neuroinflammatory response is thought to be one of the most important mediators herein (Trivedi et al., 2006).

Current best available care of SCI traumas includes early surgical intervention, hypertensive therapy with vasoactive agents, and methylprednisolone derivates, and future treatment modalities may consist of promising early phase agents such as minocycline, Cethrin™, and riluzole (Breslin and Agrawal, 2012; Cadotte and Fehlings, 2011; Hawryluk et al., 2008; Kwon et al., 2011a; Kwon et al., 2011b; Tator et al., 2012).

Mesenchymal stem cells

In the absence of full recovery for many cell degenerative disorders, stem cells may hold promise as a novel treatment modality (Donnelly et al., 2012; Sahni and Kessler, 2010). Accordingly, more than 2700 clinical cell therapy trials have been initiated during the first decade of the 21st century (Culme-Seymour et al., 2012). Stem cells are a heterogeneous group of cells, but share the unique feature of being able to self-renew as well as differentiate into more specialised cells by means of asymmetric cell division (Oliveri, 2007). Multipotent mesenchymal stem cells (MSCs) are currently occupying centre stage in preclinical and clinical stem cell research due to their plethora of regenerative effects together with their relative ease of isolation, efficient ex vivo expansion, lack of ethical concerns, and acceptable safety profile (Lalu et al., 2012; Prockop et al., 2010; Singer and Caplan, 2011; Uccelli et al., 2008; von Bahr et al., 2012). Thus, human MSCs have shown promising results in a number of diverse clinical conditions such as graft-versus-host disease (Le Blanc et al., 2008), Crohn's disease (Duijvestein et al., 2010), adjuvant induction therapy in organ transplantation (Tan et al., 2012), and ischaemic cardiomyopathy (Hare et al., 2012). MSC-like cells can be isolated from tissues such as bone marrow, adipose tissue and umbilical cord, where they are believed to reside in the perivascular niches and play a pivotal role in local tissue homeostasis (Crisan et al., 2008). Besides having intrinsic mesodermal differentiation capabilities, MSCs have more recently also shown to exhibit other coveted properties such as anti-inflammatory, immunomodulatory, anti-apoptotic, trophic and angiogenic effects. These functions are believed to be mediated by transient paracrine by-stander mechanisms and cell-to-cell contact in response to the local damaged host tissue environment rather than long-term cell engraftment and cell replacement (Caplan and Correa, 2011; Meyerrose et al., 2010; Prockop, 2007; von Bahr et al., 2012). Equally important, MSCs demonstrate pathotropism by means of chemotaxis-induced homing and migration to injured tissues following intravascular administration (Karp and Leng Teo, 2009).

MSCs have been shown to address and modulate many of the detrimental effects associated with acute and chronic damage in the traumatised spinal cord (Teixeira et al., 2013; Uccelli, 2013; Wright et al., 2011). Ameliorating effects include neuron protection from glutamate excitotoxicity (Lu et al., 2011; Uccelli et al., 2012; Voulgari-Kokota et al., 2012), reduction in levels of stress-associated proteins, pro-inflammatory cytokines and reactive oxygen species (Lanza et al., 2009; Zhou et al., 2009), inhibition of neutrophil adhesion, infiltration and respiratory burst (Pati et al., 2011; Prockop and Oh, 2012; Raffaghello et al., 2008), polarisation of classically activated pro-inflammatory M1 into an alternatively activated pro-reparatory (M2) macrophage phenotype (Giunti et al., 2012; Kim and Hematti, 2009; Nakajima et al., 2012; Zhang et al., 2010), secretion of neurotrophic factors (Crigler et al., 2006; Hawryluk et al., 2012; Li et al., 2002; Nakano et al., 2010; Zhou et al., 2009), enhancement of neural stem cell oligodendrogenic fate and remyelinisation (Inoue et al., 2003; Li et al., 2009; Rivera et al., 2006; Steffenhagen et al., 2012), function as axon guiding strands across lesion site (Hofstetter et al., 2002), reduction of cavity formation and reactive astrocyte proliferation and gliosis (Abrams et al., 2009; Voulgari-Kokota et al., 2012), and stimulation of neurite outgrowth over CSPGs, MAG and Nogo-A (Wright et al., 2007). In addition, research suggests that MSCs themselves may transdifferentiate into glial and neuronal-like cells, at least in vitro, although the exact transdifferentiation potential in vivo remains debated (Chen et al., 2006; Krabbe et al., 2005). Taken together, these observations suggest that administration of MSCs may lead to a net beneficial effect in the recovery process following SCI secondary damage.

Objective

A small number of human studies have investigated the use of MSCs in SCI patients. They have mainly focused on safety and feasibility issues and have lacked a proper randomised control group and have been underpowered. By contrast, an increasing number of rodent studies have during the last decade investigated the efficacy of MSCs on locomotor recovery, a relevant clinical outcome which is considered important among SCI patients (Kwon et al., 2012; Simpson et al., 2012).

Meta-analyses of controlled studies increase the power and precision of the estimated intervention effect and therefore yield a more powerful test of the null hypothesis than any of the separate studies (Higgins and Green, 2008). However, to our knowledge, no quantitative data synthesis of a stem cell therapy for SCI exists. We therefore, systematically reviewed and meta-analyzed studies that assessed the efficacy of MSCs versus control (placebo or no treatment) in an established and widely used animal model of traumatic SCI. Our aim was to determine whether MSCs improved locomotor recovery and to use meta-analysis and meta-regression to explore for variations in effect size. Finally, we subjected the studies to an assessment of bias to exclude systematic errors and to an assessment of risks of play of chance to exclude random errors.

Material and methods

Eligibility criteria

Types of studies: Controlled studies assessing the administration of MSCs to rats with traumatic SCI. No language, publication date, or publication status restrictions were imposed.

Types of participants: Laboratory rats of any age, gender or strain exposed to traumatic SCI induced by contusion or compression. Laceration/transection models of SCI were excluded as this model does not represent the typical crush injury mechanism in humans and has limited rostro-caudal secondary damage spread (Beattie and Bresnahan, 2000; Dietz and Curt, 2006).

Types of intervention: Plastic-adherent, ex vivo expanded MSCs (Dominici et al., 2006) irrespective of donor species or tissue origin. MSCs substantially manipulated, e.g., into neuron-like cells (differentiation cocktails, transfection) were excluded whereas more basic cell culturing steps maintaining overall MSC phenotype were allowed (e.g., activation or 'licensing' with IFNγ). Labeling or transfection with markers for cellular tracing and imaging (green fluorescent protein, lacZ, bromodeoxyuridine, superparamagnetic iron oxide particles etc.) were included. Co-culture or concomitant injection with other cell types or use of adjuvant products (e.g., matrices, scaffolding), whether these were used for seeding prior or during transplantation or used to recruit MSCs in vivo, were excluded. Control interventions consisted of placebo (saline, culture medium or similar vehicle) or no treatment.

Type of outcome measure: Behavioural assessment measured by the Basso–Beattie–Bresnahan (BBB) locomotor rating scale (Basso et al., 1995), which is a well-documented and widely used ordinal (i.e. non-linear) scale with discrete values ranging from 0 (no observable hind limb movement) to 21 (coordinated gait, consistent toe clearance, predominant paw position is parallel throughout stance, consistent trunk stability, and tail is consistently up). It subjectively documents limb movements and walking characteristics in an open-field environment and is a useful indicator of the basic overground locomotion of the animal and whether or not the animal would be capable of performing more difficult motor-related assessment tests that require specific abilities (Burke and Magnuson, 2012).

Information sources, literature search and study selection

Studies were identified by searching bibliographic databases, scanning reference lists of articles, and hand-searching. No limits were applied for language and non-English papers were translated. We developed a sensitive literature search strategy (Table 1) and our search in bibliographic databases was applied to MEDLINE and EMBASE (dates of inception to 1 Nov 2012). Titles and abstracts of identified references were screened for eligibility and clearly irrelevant references were excluded. Remaining references underwent full-paper assessment. Final eligibility assessment was performed by one skilled reviewer (RSO) with particular knowledge of isolation, culture and characterisation of MSCs.

Data collection process

We sought to avoid double counting and pieced together data from multiple reports of the same study by, e.g., juxtaposing author names, treatment comparisons, sample sizes, or outcomes. We contacted corresponding investigators by e-mail to obtain additional information not clearly reported in the reference. If investigators did not respond within two weeks, their reference was excluded from quantitative syntheses if missing items necessitated calculation of summary effect measures. If a corresponding investigator did not provide information on a putative explanatory variable, the study was still included in the primary meta-analysis but excluded from descriptive analyses and the relevant subgroup analyses and meta-regressions.

Table 1
Search terms used in the bibliographic databases MEDLINE and EMBASE (searched from dates of inception to 1 Nov 2012).

Database	Search terms
MEDLINE (PubMed)	(mesenchymal stem cells [mh] OR mesenchymal stromal cells [mh] OR mesenchymal stem cell* [tiab] OR mesenchymal stromal cell* [tiab] OR marrow stromal cell* [tiab] OR bone marrow stem cell*[tiab] OR bone marrow-derived stromal cell* [tiab] OR mesenchymal precursor cell* OR MSCs [tiab] OR MSC [tiab] OR BMSCs [tiab] OR BMSC [tiab] OR wharton jelly [mh] OR wharton's jelly [tiab] or umbilical cord stroma [tiab] OR fetal blood [mh] OR umbilical cord blood [tiab] OR UCB [tiab] OR adipose-derived stromal cell* [tiab] OR ASCs [tiab] OR adipose stem cell* [tiab] OR pre-adipocyte* [tiab] OR stromal vascular fraction stem cell* OR adipose tissue-derived stem cell* OR ASC [tiab] OR dental pulp [tiab]) AND (spinal cord injuries [mh] OR spinal cord injury [tiab] OR spinal cord injuries [tiab] OR spinal cord contusion [tiab] OR spinal cord transection [tiab] OR injured spinal cord [tiab])
EMBASE	(mesenchymal stem cells.mp. OR mesenchymal stem cell/OR mesenchymal stromal cells.mp. or mesenchymal stroma cell/OR bone marrow stromal cells.mp.) AND (spinal cord injury.mp. or spinal cord injury/)

Data items

The following items decided a priori were extracted from each included study: reference details (journal name, publication year, and name, contact information and affiliation of the corresponding investigator); recipient animal (rat strain, sex, average weight); SCI (traumatic model and affected spinal cord segment); MSCs (donor species and tissue source); intervention regime (time from SCI to intervention, administration route, number of injections, and total dose); use of immunosuppressive agent; methodological quality (method of generation of allocation sequence, method of allocation concealment, blinding of study personnel or outcome assessors, and considerations on sample size or study power). From the primary extracted data we stratified the country of origin according to socio-economic development and longstanding tradition in modern biomedical research (Panagiotou et al., 2013), calculated mean relative MSC dose (cells/kg), and classified the graft type according to donor-recipient similarity (i.e. autologous, syngeneic, allogeneic, or xenogeneic).

Risk of bias in individual studies

To ascertain quality components associated with spurious treatment effect in clinical trials (Hrobjartsson et al., 2013; Savovic et al., 2012; Wood et al., 2008), two reviewers (RSO and SB) working independently and with adequate reliability determined the adequacy of randomisation and concealment of allocation, blinding of study personnel and outcome assessors, and registered sample size estimations or power calculations. Disagreements between the reviewers were resolved by consensus.

Summary effect measure

Summary effect measure was the difference in BBB score means between MSC group and control at maximal follow-up. To calculate this we extracted for each group: mean BBB score, standard deviation (SD) and group size. Where a mean BBB score was measured serially, only the final measure was used. If BBB scores were reported for each hind leg, an average value was calculated. In studies including both a placebo group and a no-treatment group, the two mean BBB scores and SDs were averaged, respectively, and the group sizes were added up (Higgins and Deeks, 2008). If an SD was not reported directly, it was calculated by multiplying the reported standard error (SE) with the square root of the group size. If numbers necessary for the calculation of summary effect measures were not adequately reported in the main text, data points were extracted from published graphs and recorded using graph digitizing software (GetData Graph Digitizer 2.24; S. Fedorov; http://getdata-graph-digitizer.com).

Synthesis of results

The meta-analysed difference of BBB score means with 95% confidence interval (CI) was calculated using the random-effects model in anticipation of significant experimental diversity between studies (DerSimonian and Laird, 1986). A random-effects model suggests the expected average BBB improvement of all samples of individual true effect sizes and not a single common true effect size from studies dispersed due to sampling error. Statistical heterogeneity was explored using the inconsistency (I^2) measure (Higgins and Thompson, 2002).

For references with two or more separate MSC groups compared against a common control group (e.g. different administration route or dose), the control group size was divided equally among the number of MSC groups (Higgins et al., 2008).

Risk of bias across studies

To assess the possibility of publication bias ('the file drawer effect') and other reporting biases we plotted the difference of BBB score means against its precision, i.e. 1/standard error of BBB difference (Egger et al., 1997). The level of asymmetry of this 'funnel plot' was assessed with Egger's linear regression method (Egger et al., 1997) and Duval and Tweedie's trim-and-fill method (Duval and Tweedie, 2000).

Additional analyses

Cumulative meta-analysis was performed in order to establish approximate publication year for evidence of effect. However, since meta-analyses are prone to inflated random errors (both type I and type II errors) due to sparse data and repeated significance testing as study data accumulate, we also applied trial sequential analysis (TSA) in order to confirm the results of the meta-analyses (Wetterslev et al., 2008, 2009). TSA adjusts for random-error risk and provides a conservative estimate of the required number of study subjects (information size) in order to carry out a definitive meta-analysis. Meta-analyses not reaching the required information size are analysed with monitoring boundaries analogous to interim monitoring boundaries in a single clinical trial (Brok et al., 2008; Thorlund et al., 2009). We calculated the required information size based on a median final mean BBB score in the control group, a minimal relevant difference of mean BBB score approximately corresponding to the lower confidence limit of the intervention effect size yielded by the primary meta-analysis, an overall significance level (α) of 0.05, a type II error (β) risk of 0.1 (i.e., power of 90%), and a diversity (D^2) estimated empirically by the TSA software (Wetterslev et al., 2009). Next, to adjust for between-study heterogeneity, the required information size was then corrected with the factor $1/(1-D^2)$ to yield a final diversity-adjusted required information size. A cumulative Z-curve was constructed by including each study according to the order of their publication year. If more studies were published the same year, we analysed studies in alphabetical order, according to the last name of the first author. We assessed the crossing of the cumulative Z-curve of the discrete monitoring boundaries based on the O'Brien–Fleming type α-spending function (O'Brien and Fleming, 1979), which is relatively insensitive to the number of repeated significance tests (Thorlund et al., 2011).

Subgroup analyses were performed with stratification according to a number of pre-specified categorical explanatory variables, and differences between subgroups were evaluated with the Q-test for heterogeneity based on a random-effects model with pooled estimate of τ^2 (Borenstein et al., 2009b).

Univariate random-effects meta-regression was performed in order to explore the potential effect of a number of pre-specified continuous explanatory variables using the method of moments (Borenstein et al, 2009a).

Potential explanatory variables were included in a multivariate random-effects meta-regression model to adjust for spurious statistical significance (Berkey et al., 1995; Harbord and Higgins, 2008; Knapp and Hartung, 2003). We did not allow the number of explanatory variables to exceed a ratio of 1:10 compared to the number of studies (Deeks et al., 2008; Higgins and Thompson, 2002).

Statistics

Descriptive statistics were performed with SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). Funnel plot asymmetry assessment, meta-analyses, testing for differences between subgroups, and continuous univariate meta-regressions were conducted with Comprehensive Meta Analysis 2.2 (Biostat Inc., Englewood, NJ, USA). TSA was conducted with TSA Viewer 0.9 beta (Copenhagen Trial Unit, Copenhagen, Denmark, www.ctu.dk/tsa). Multivariate meta-regression was conducted with Stata 12.0 (StataCorp LP, College Station, TX, USA). A two-sided P-value < 0.05 was considered statistically significant except for TSAs where significance level varied according to the calculated O'Brien–Fleming type α-spending function.

Results

Study selection

We identified 83 eligible studies/comparisons reported in 61 references comprising a total of 1,568 rats to be included in our main meta-analysis (Fig. 1; Table S1). A further 24 studies reported in 17 references were considered eligible but did not report the necessary data for the calculation of the summary effect measures and were therefore excluded from further analyses (Table S2). Of those 24 studies, 22 reported a beneficial effect of MSCs.

Study characteristics

Table S3 shows the characteristics of the included studies. The median year of publication was 2010 (range of 2000 to 2012). Median sample size for all 83 studies was 16 rats (range of 8 to 43). Eleven studies (13%) reported considerations of sample size but none provided exact details on sample size calculations. Forty-nine studies (59%) were from less developed countries, eight studies (10%) were from countries in transition, and 26 studies (31%) were from more developed countries (Panagiotou et al., 2013). China was the single biggest contributor reporting almost one third (31%) of all studies. Sixteen studies were published in non-English journals.

Bone marrow was the most frequently used MSC tissue source (84%). Allogeneic and xenogeneic MSCs were predominant graft types (57%) and human MSCs were used in 30 (36%) studies. Of 80 studies using allogeneic or xenogeneic MSCs, 22 (28%) reported the use of an immunosuppressive agent (predominantly cyclosporin) following transplantation.

Four different rat strains were used as recipients with Sprague–Dawley (46%) and Wistar (49%) rats comprising the vast majority.

Contusion lesion was used in 78% compared to compression. All studies except one (spinal cord level L1) reported induction of SCI in the thoracic region (range T6 to T12) with spinal cord levels T8 to T10 comprising 68%. Thus, no study investigated a cervical SCI model.

Median time from SCI to intervention was 7 days (range of 0 to 90), and median time from intervention to final BBB score assessment was 35 days (range of 21 to 365).

Intraparenchymal injection (64%) was the most frequently reported administration route (median total dose of 5×10^5 MSCs; range of 3×10^3 to 5×10^6). Median total dose for intrathecal injection (17%) was 1.25×10^6 MSCs (range of 1×10^5 to 5×10^6) whereas for the remaining studies intravenous (17%) median total dose was 1×10^5 MSCs (range of 1×10^3 to 9×10^6), which corresponded to a relative dose of 4.2×10^6 MSCs/kg (range of 3.1×10^6 to 3.3×10^7). Two studies reported intra-ventricular administration by means of drilled transcranial route.

Risk of bias within studies

Fifty-three studies comprising 1,047 rats (67%) were labelled as randomised. Of these, four studies ($I^2 = 93\%$) reported adequate randomisation procedure (i.e. both adequate generation of allocation sequence and adequate allocation concealment) whereas the other 49 studies ($I^2 = 98\%$) had unclear randomisation procedure. The remaining 30 studies ($I^2 = 99\%$) comprising 521 rats (33%) did mention randomisation at all. No study reported blinding of study personnel

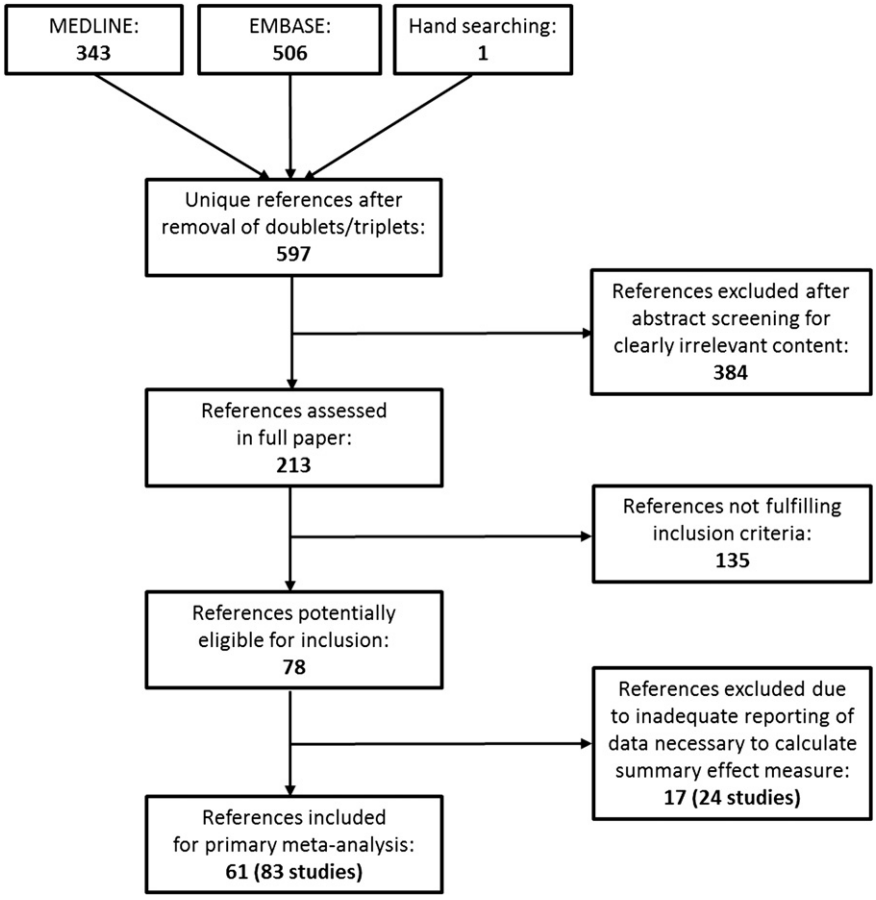

Fig. 1. Flow chart illustrating the literature search strategy and the different phases of study eligibility assessment. Bibliographic databases were searched from dates of inception to 1 November 2012.

whereas 48 studies comprising 891 rats (57%) reported use of blinded outcome assessment. Four studies had both adequate randomisation and blinded outcome assessment.

Meta-analysis of all studies

Fig. 2 shows a forest plot of the primary random-effects meta-analysis of all 83 eligible studies. There was high between-study heterogeneity ($I^2 = 98.9\%$). Pooled difference in BBB score means was 3.9 (95% CI 3.1 to 4.7; $P < 0.001$) in favour of MSCs. No study reported a statistically significant detrimental effect.

Risk of bias across studies

Fig. 3 shows funnel plots of the differences in mean BBB scores against their precision (inverse SE). Neither Egger's linear regression method (intercept 0.49; 95% CI −2.49 to 3.48; $P = 0.74$) nor Duval and Tweedie's trim-and-fill method suggested the presence of unidentified negative studies (Fig. 3). Separate funnel plots and formal testing stratified according to randomisation (adequate, unclear or no description) did not indicate publication bias of negative studies, but again of missing positive studies (data not shown).

Additional analyses

Fig. 4 shows a plot of the cumulative meta-analysis stratified according to publication year, without adjustment for repeated significance testing. The figure suggests that sufficient evidence of the beneficial effect of MSCs on locomotor recovery began to accumulate from 2006 onwards.

Based upon an estimated median value of 8.5 of the mean BBB scores in the control groups, a difference in mean BBB score of 3.0 (approximately corresponding to the lower 95% confidence limit from the primary meta-analysis), an overall significance level (α) of 0.05, a type II error risk (β) of 0.1 (i.e., power 90%), and diversity (D^2) of 100% in all studies in a random-effects model meta-analysis, we calculated that the diversity-adjusted required information size to detect or reject such an intervention effect as 859 rats (Fig. 5). The cumulative Z-score crossed the upper monitoring boundary for benefit after the 19th study (published in 2007) or 389 accrued rats with a continuing upward slope, confirming a beneficial effect of MSCs on locomotor recovery if one disregards the risks of bias (Fig. 5A). We then repeated TSA using a minimal relevant difference of 1.0 (i.e., the lowest possible discrete value on the BBB scale) which yielded a diversity-adjusted required information size of 7087 rats. The trial sequential monitoring boundary for benefit was this time crossed by the cumulative Z-score after the 66th study (published in 2011) or 1243 accrued rats (Fig. 5B) thus confirming adequate study power to detect the smallest possible intervention effect size on the BBB scale.

Subgroup analyses (Fig. 6) suggested that locomotor recovery was associated with graft type ($P < 0.001$), recipient rat strain ($P < 0.001$), SCI trauma model ($P = 0.017$), immunosuppressive status in allogeneic or xenogeneic grafting ($P = 0.047$) and blinded outcome assessment ($P = 0.042$), but not with adequacy of randomisation procedure ($P = 0.614$), MSC donor tissue ($P = 0.636$), administration route ($P = 0.624$), described as randomised ($P = 0.341$), or national level of longstanding research tradition ($P = 0.159$). Continuous univariate meta-regression suggested that an increase in difference in BBB score increased as time from SCI to intervention increased ($P < 0.001$). When stratifying relative cell dose according to administration route, increased

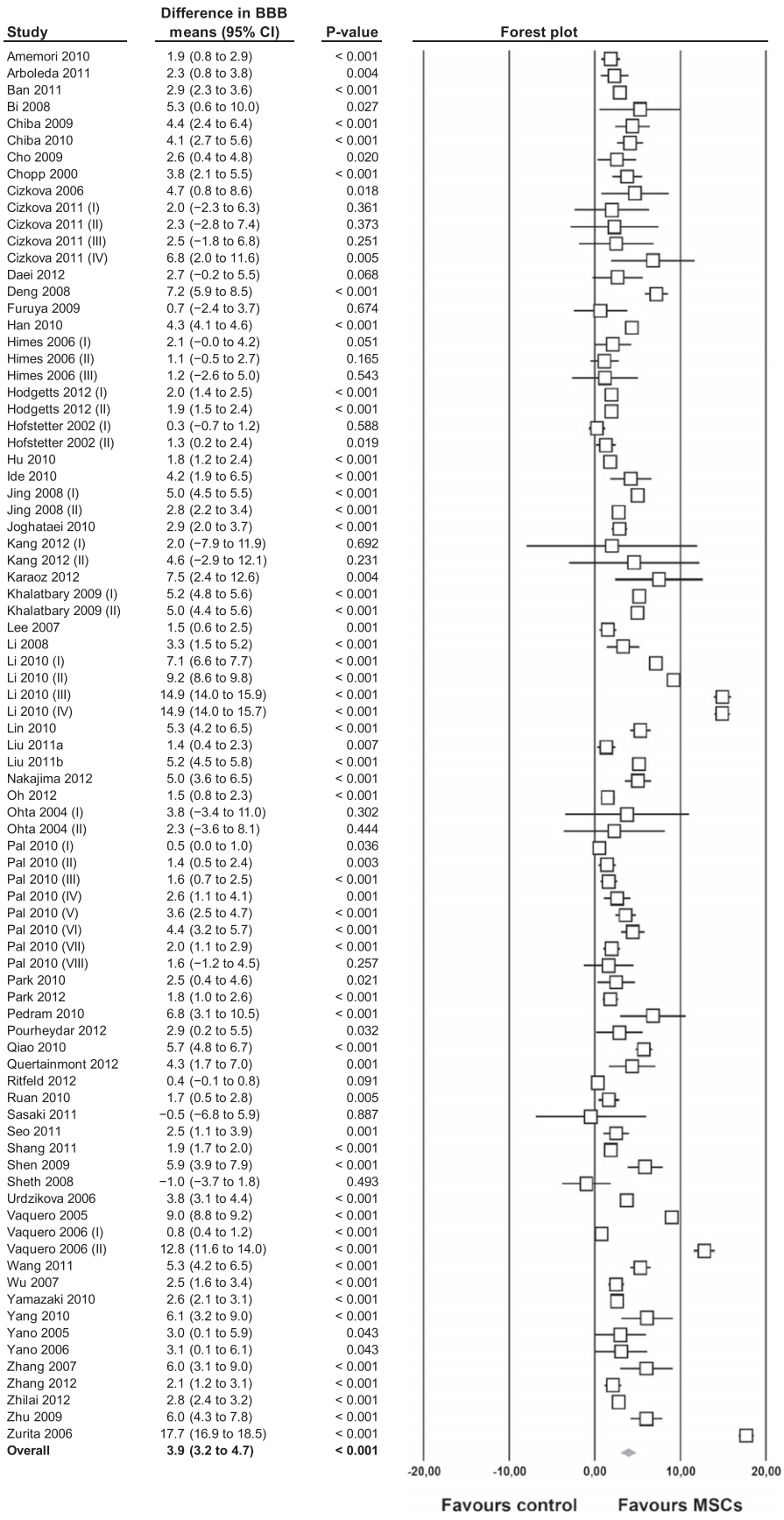

Fig. 2. Forest plot of a random-effects meta-analysis of 83 eligible studies (comprising 1568 rats) comparing MSCs versus controls on locomotor recovery after traumatic SCI. Results from each study are displayed as a square and a horizontal line, representing the intervention effect estimate (difference in BBB score means) together with its 95% CI. The combined random-effects estimate (3.9) and its 95% CI (3.1 to 4.7) are represented by a diamond at the bottom. Roman numerals in brackets after author name and year represent separate eligible studies/comparisons from within the same bibliographic reference.

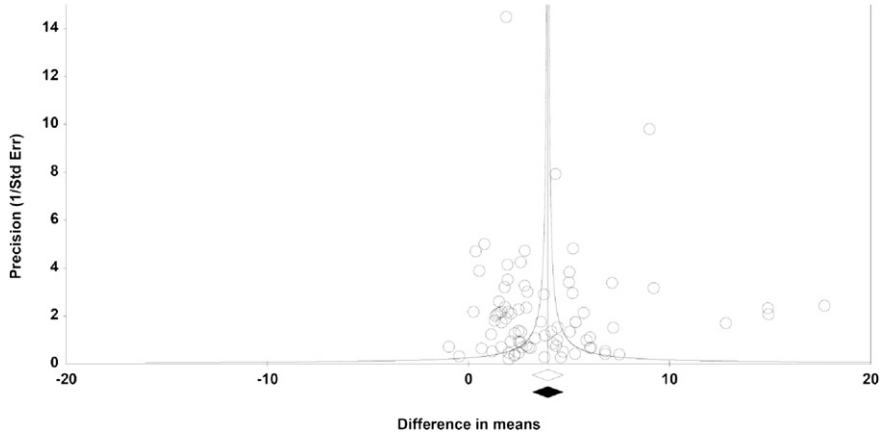

Fig. 3. Funnel plot of the differences in BBB score means against their precision (inverse standard error). The open diamond represents the pooled random-effects intervention effect size with 95% CI. Formal assessment with Duval and Tweedie's trim-and-fill method does not add any imputed studies on left side of the plot suggesting that no negative studies remain unidentified and the overall intervention effect size (filled diamond) remains unchanged.

intervention effect size was positively correlated with relative cell dose for intraparenchymal ($P < 0.001$) and intrathecal administration route ($P < 0.001$) but negatively correlated with cell number for intravenous route ($P < 0.001$). Effect size was positively correlated to time from intervention to final BBB score assessment ($P < 0.001$). However, after adjustment in a multivariate random-effects meta-regression, only time from intervention to final BBB score assessment remained significantly associated with locomotor recovery ($P = 0.022$).

Discussion

It has been forcefully argued that all reports of new interventions should begin with and end with systematic reviews (Chalmers, 2009; Clarke et al., 2007), and a number of cases have illustrated that omission may have serious consequences for public health (Crowley et al., 1990; Fergusson et al., 2005; Gilbert et al., 2005; Teo et al., 1993). Notably, translational stem cell research has become a particularly sensitive research area because many 'treatments' that lack any evidence of beneficial effect are currently being offered to vulnerable groups of patients (Blight et al., 2009; Regenberg et al., 2009). The key advantage of a systematic approach of a meta-analysis is that all steps are clearly described so that the process is fully transparent. Accordingly, systematic reviews and meta-analyses of animal studies offer a sensible and rational approach to assess the translational potential of promising experimental interventions before decisions are made to proceed with clinical trials. The present review is, to our knowledge, the first attempt to systematically collect all available evidence and critically assess and quantify the efficacy of a stem cell population in a widely used preclinical model of traumatic SCI.

Summary of evidence

Using a sensitive and exhaustive literature search strategy we identified more than 80 eligible studies. Our random-effects meta-analysis suggested a beneficial effect of MSCs, which TSA confirmed using highly conservative monitoring boundaries for statistical significance. The use of a random-effects model suggests the expected average BBB improvement of all samples of individual true effect sizes and not a single common true effect size from studies dispersed due to sampling error. However, to extrapolate the exact size of the observed BBB difference into a human clinical setting would be nonsensical. Furthermore, in accepting these effects one has to disregard the substantial risks of bias that the included studies may suffer from. On the other hand, we could not demonstrate an effect of bias risks on the estimated intervention effect.

Some studies reported very large treatment effects reflecting very limited spontaneous recovery in the control group (e.g. Vaquero et al., 2006). We do not have an explanation for such findings other than the use of a much more severe contusion model with release of the impactor from a much greater height, perhaps in combination with late intervention. However, we do not think it would be appropriate to exclude such studies with 'outlying' data based on post-hoc considerations.

Cumulative meta-analysis suggested that evidence of a beneficial effect began to accumulate from 2006 onwards. Similarly, our TSA showed that such an effect existed (without risks of random errors) by 2007 based upon a minimal relevant difference of 3.0 in the BBB score (or by 2011 based upon a minimal relevant difference of 1.0), thus suggesting that a substantial number of rat SCI studies may have been conducted to limited avail.

Statistical heterogeneity and explanatory variables

As anticipated, we observed substantial between-study heterogeneity on treatment effect. Heterogeneity not explained by random error is a consequence of experimental diversity between studies (Deeks et al., 2008). The observed heterogeneity was so substantial that it may be questioned if it is meaningful to conduct a meta-analysis. On the other hand, meta-analytic subgroup analyses can investigate heterogeneous results or answer specific questions about experimental groups, intervention scheme, or types of study. Our pre-specified subgroup analyses and univariate meta-regression suggested that the effect of MSCs on locomotor recovery could be associated with some explanatory variables. However, it is important to keep in mind that such analyses are entirely observational by nature and not based on randomised comparisons. Hence, they suffer the usual limitations of any observational investigation, including bias through confounding by other potential explanatory variables. Importantly, subgroup analyses can only be hypothesis generating and not hypothesis confirmatory (Deeks et al., 2008). We also conducted a multivariate random-effects meta-regression to allow the effects of potential explanatory variables to be investigated simultaneously (Thompson and Higgins, 2002). However, we found that a positive correlation between time from intervention to final BBB score assessment and locomotor recovery was the only explanatory variable that remained statistically significant. This observation may be explained by the fact that a substantial number of studies conducted the final BBB score assessment before reaching the plateau recovery phase.

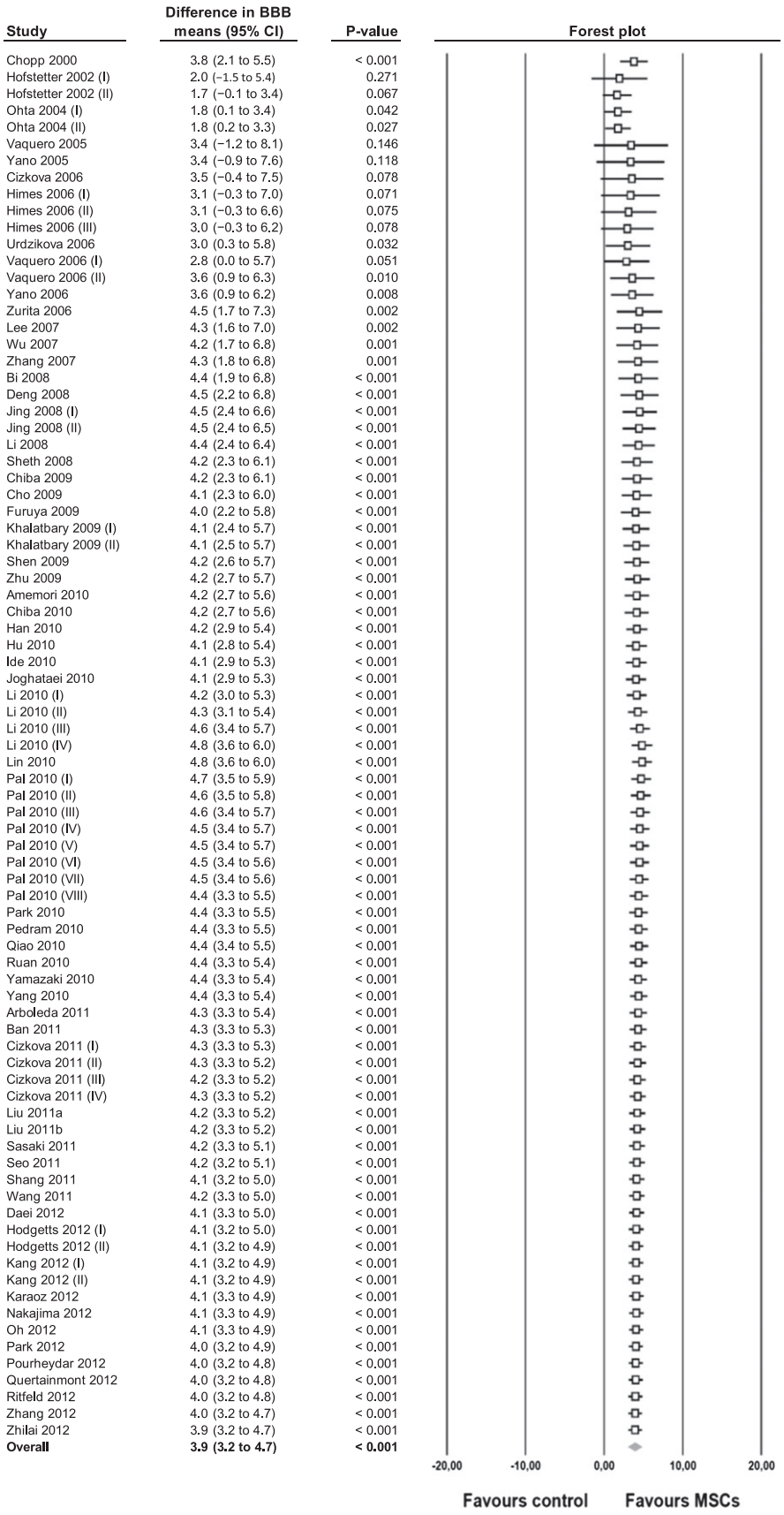

Fig. 4. Cumulative forest plot of meta-analyses with individual studies added in a stepwise manner according to publication year and surname of the first author. Results from each cumulative meta-analysis are displayed as a square and a horizontal line, representing the treatment effect estimate together with its 95% CI. Thus, the treatment effect plotted next to each study name represents the *accumulated* pooled treatment effect of this study combined with all the studies ranked above it. As the data accumulate (i.e. increasing sample size) the treatment effect steadily begins to oscillate to a lesser degree and the 95% CI becomes more narrow after a certain point of accumulated data. The data indicate that evidence of beneficial effect of MSCs on locomotor recovery began to accumulate from 2006 onwards.

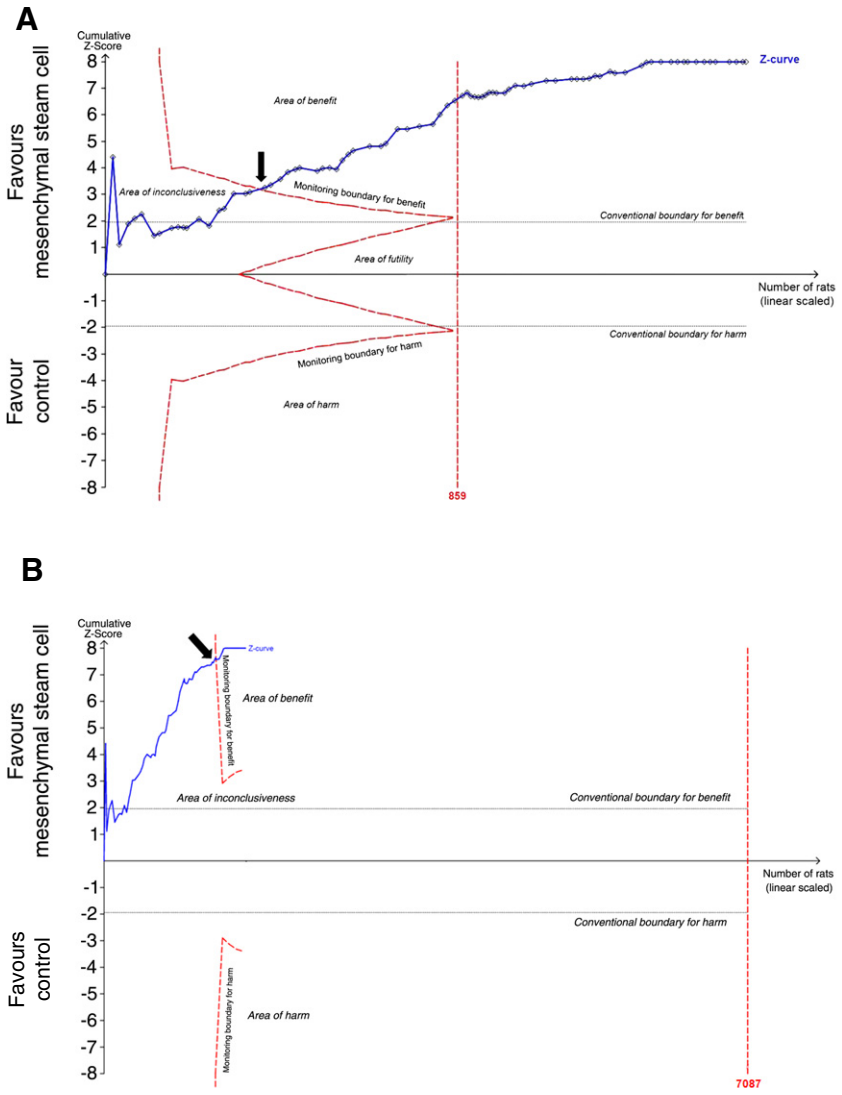

Fig. 5. TSAs of the effect of MSCs on locomotor recovery after SCI. (A) The diversity-adjusted required information size is based on a median value of mean BBB scores of 8.5 in the control groups, a minimum relevant difference in mean BBB score of 3.0, an overall significance level (α) of 0.05, a type II risk (β) of 0.1 (i.e. power 90%), a diversity (D) of 100% and equals 859 rats (vertical dotted red line). The cumulative Z-curve (solid blue line) connected by individual studies (small diamonds) crosses the upper O'Brien–Fleming monitoring boundary of benefit (descending dotted red line) after the 19th study (389 accrued rats) at a cumulative Z-score of 3.24 ($P = 0.0012$) (black arrow). Total number of accrued rats (as of date of literature search) is 1568 rats, corresponding to 183% of the diversity-adjusted required information size. (B) The diversity-adjusted required information size is based on a median value of mean BBB scores of 8.5 in the control groups, a minimum relevant difference in mean BBB score of 1.0, an overall significance level (α) of 0.05, a type II risk (β) of 0.1 (ie. power 90%), a diversity (D) of 100% and equals 8,087 rats (vertical dotted red line). The cumulative Z-curve (solid blue line) connected by individual studies (omitted for clarity) crosses the upper O'Brien–Fleming monitoring boundary of benefit (descending dotted red line) after the 66th study (1243 accrued rats) at a cumulative Z-score of 7.57 ($P < 0.0001$) (black arrow). Total number of accrued rats (as of date of literature search) is 1568 rats, corresponding to 22% of the diversity-adjusted required information size (7087 rats).

Bias risk

Reporting of methodological components, such as randomisation and blinding, was generally poor and therefore limits the assessment of risk of bias of the included studies. Only four studies reported both adequate method of generation of allocation sequence and adequate allocation concealment. These studies also implemented the use of a placebo, which reduces the risk of assessment bias. Furthermore, no study reported blinding of study personnel, and only 48 (58%) studies reported blinding of outcome assessors, which is in accordance with the findings of others (Perel et al., 2007). However, stratification according to methodological components did not show any significant association with treatment effect after adjustment in a meta-regression nor suggest publication bias against negative studies using trim-and-fill. However, the lack of demonstrable association between potential explanatory variables and treatment effect could be due to inadequate meta-analytic study power.

MSC tissue source

We could not detect any clear association between locomotor recovery and MSCs isolated from specific tissues. Bone marrow and adipose tissue (and to a lesser extent umbilical cord stroma) have been the traditional tissue sources of MSCs in clinical stem cell research. Although MSCs from different tissues share certain characteristics of morphology, plastic adherence and differentiation potential (Dominici et al., 2006), it has become evident that there are subtle but distinct tissue differences in, e.g., expression of surface markers, which could be important for the design of MSC-based interventions (Heazlewood and Atkinson, 2013). Bone marrow-derived MSCs have remained the 'gold standard' having been most thoroughly characterised in preclinical and clinical research. Bone marrow-derived MSCs can be easily isolated from the iliac crest and efficiently ex vivo expanded to several hundred millions cells within a relatively short period of time (Bartmann et al., 2007; Schallmoser et al., 2008). Initial isolation and

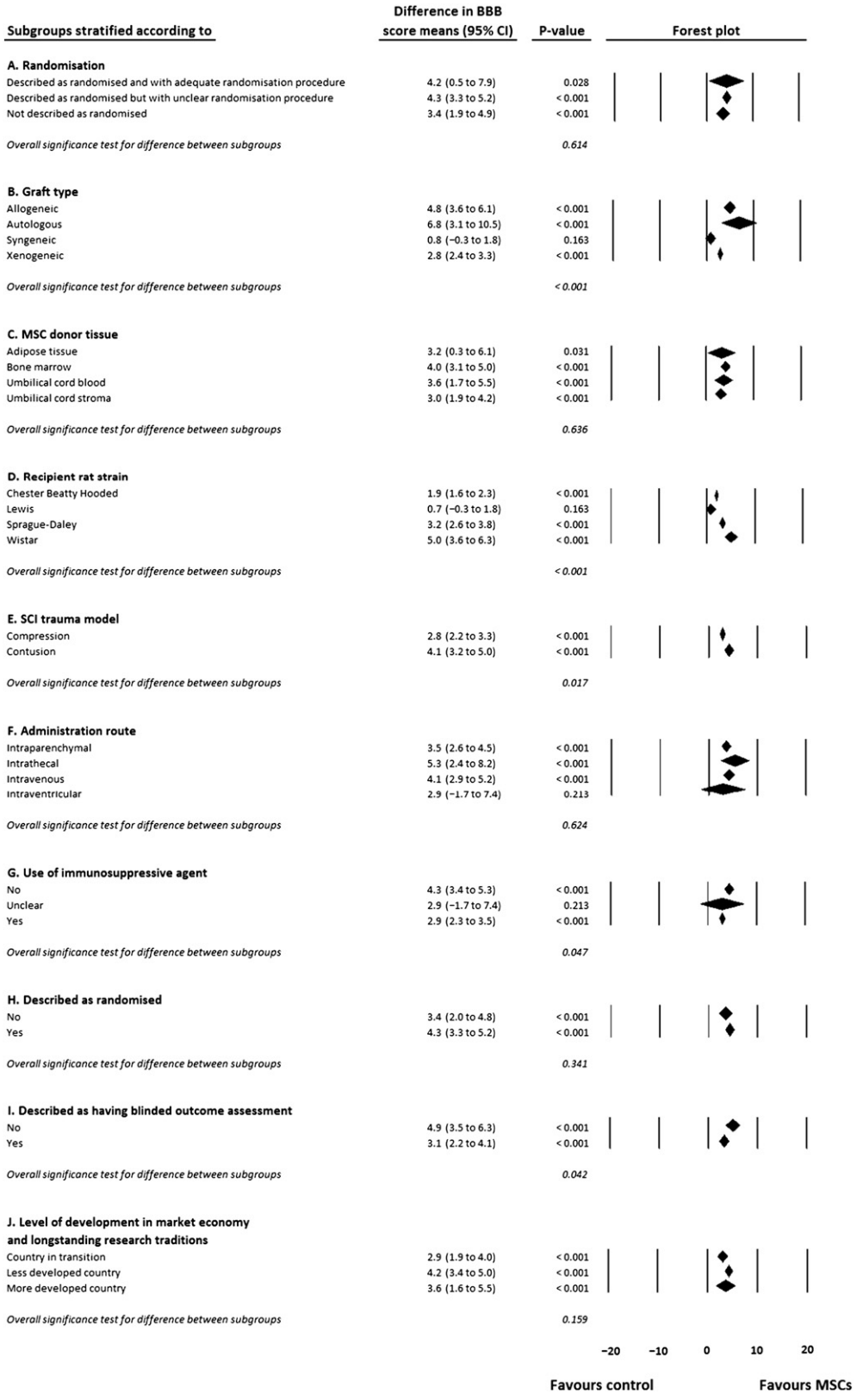

Fig. 6. Forest plots of subgroup analyses stratified according to a number of potential explanatory variables. The individual studies have been omitted from the plots for clarity and only the combined random-effects estimate with corresponding 95% CI for each subgroup is presented (diamonds). Abbreviations: MSC = mesenchymal stem cell; CI = confidence interval.

purging of adipose tissue-derived MSCs can be somewhat more cumbersome compared to bone marrow-derived MSCs, although initial cell number in the adipose stromal vascular fraction, proliferation rates and final yield can be very high (Trojahn Kølle et al., 2013). MSCs from umbilical cord stroma may represent a distinct population of 'pristine' MSCs ontogenetically situated between more lineage-restricted adult

MSCs and pluripotent embryonic stem cells. Cell harvesting from the umbilical cord stroma is non-invasive and accordingly does not pose any ethical challenges, since this tissue is otherwise discarded. Notably, the cells could have superior immunosuppressive effects compared to adult tissue sources (Manochantr et al., 2013; Najar et al., 2012).

Graft type

Immunosuppressive agents reduce secondary damage and may improve outcomes following SCI (Diaz-Ruiz et al., 1999, 2004; Ibarra et al., 2003), although there are rat-strain specific differences in responses (Cui et al., 2007). Hence, the concomitant use of immunosuppressive agents in rats transplanted with MSCs could explain some of the observed effect in locomotor recovery. However, we could not detect any increased locomotor recovery associated with the use immunosuppression in our subgroup analysis of studies using allogeneic or xenogeneic grafts. In a direct head-to-head comparison Hodgetts et al. did not detect any differences in locomotor recovery using cyclosporin (Hodgetts et al., in press). The hypo-immunogenic properties of MSCs and lack of long-term engraftment could be plausible explanations for this observation.

Timing

The development of secondary spinal cord damage follows a distinct timeframe, and in for example neonatal hypoxic–ischaemic encephalopathy timing of interventional hypothermia has been shown to be crucial (Jacobs et al., 2013). However, after adjusting for other explanatory variables, we were not able to detect any clear association between locomotor recovery and timing of MSC administration. However, this finding could be due to lack of accumulated study power and it should be noted that one uneligible study assessing different timing schedules would seem to favour early (<72 h) MSC intervention in the rat model (Osaka et al., 2010).

Administration route

Because recovery from SCI may depend on the preservation of remaining axons and neurons in the injury region, it is essential that these not be further depleted by procedures aimed at repair. We were not able to detect any statistically significant differences on locomotor recovery between intraparenchymal, intrathecal or intravenous administration. Three included studies specifically compared intraparenchymal and intravenous route (Jing et al., 2008; Kang et al., 2012; Khalatbary and Tiraihi, 2009). Interestingly, intravenous administration did not perform inferior to intraparenchymal with two of the studies using the same cell dose for both routes (Jing et al., 2008; Kang et al., 2012). Similar observations has been made by Morando et al. comparing intravenous and intrathecal MSC injection in an animal model of multiple sclerosis (Morando et al., 2012). By contrast, Vaquero et al. observed no effect of intravenous administered MSCs compared to intraparenchymal route, which may be explained by their use of a severe force of contusion combined with late intervention (Vaquero et al., 2006). Finally, in a recent study on a chronic model of SCI by Kim et al. (published after our literature search) both intravenously and intraparenchymal administration of the same amount of MSCs resulted in similar locomotor recovery despite fewer numbers of observable engrafted cells in the intravenous group (Kim et al., 2013).

The local MSC concentration necessary for an observable beneficial effect may be relatively low in the damaged spinal cord area, thus facilitating efficient autologous MSC ex vivo expansion and subsequent local administration. On the other hand SCI poses substantial challenges to intraparenchymal injection due to pre-existing tissue injury, relatively large injection volumes, high cell concentrations, and high delivery rates. Current techniques are mainly limited to a single injection during an open surgical exposure. One difficulty is that methods to observe injectate dispersion and monitor tissue pressure during injection have not been thoroughly established. This has raised concerns that intraparenchymal injections may cause further tissue injury that varies from minor to severe (e.g. needle trauma). Injectate backout or extrusion, which has previously been considered a technical failure, may be argued as being a clear indicator of excessive intraparenchymal pressure (Guest et al., 2011). Accordingly, pressure based injections of large volumes may be damaging because the tissue tolerances may be exceeded by raised intraparenchymal pressures leading to hydrodynamic dissection and possible ischemia (Guest et al., 2011).

Intrathecal administration has been proposed as less invasive method (Bakshi et al., 2004). The technique is well suited to multiple injections over an extended time period and it may be possible to use intrathecal catheter techniques to make injections closer to the target site, thereby perhaps increasing local MSC concentration. On the other hand, it is perhaps less likely that transplanted MSCs will efficiently home to sites of chronic disease due to lacking inflammatory cell activity, dysfunctional subarachnoid space, and occlusion by scarring.

The advantages of intravenous administration include lack of need of an operating theatre or procedural imaging combined with extensive clinical experience on thousands of patients since the first MSC infusions in the 1990s. In a recent systematic review and meta-analysis of 36 clinical trials comprising 1,012 patients there were no development of acute infusional toxicity, organ system complications, infection, or longer term adverse events (death, malignancy) associated with intravenous MSC administration (Lalu et al., 2012). An increased risk of transient fever was reported, not unlike similar reactions occasionally observed with red blood cell transfusions, but this was not associated with any long term sequelae. The putative low fraction of the infused MSCs reaching target lesion site could be a major obstacle for intravenous delivery since the majority of MSCs are trapped within the lungs during pulmonary passage due to their size, adhesion molecules and possibly deformability (Fischer et al., 2009). However, pulmonary trapping seems to be a transient phenomenon, and MSC can exert therapeutic plasticity even if not migrating completely to the lesion site due to secretion of factors or cross-talking with immune cells in regional lymph nodes (Kim et al., 2013; Uccelli, 2013). Other potential disadvantages of intravascular administration include risk of instant blood-mediated inflammatory reaction after blood exposure due to prothrombotic factors on the MSC surface which may compromise cell survival, engraftment, and function (Moll et al., 2012). However, low-passage MSCs, as typically used in clinical applications, show only a weak triggering at the currently applied clinical doses (1 to 3×10^6 MSC/kg) (Moll et al., 2012).

Dose

Median relative MSC dose was 4.2×10^6 MSC/kg for rats receiving systemic infusion. This is somewhat more than standard doses currently used in clinical trials (1 to 3×10^6 MSC/kg) yet manageable to manufacture and relative doses of up to 10×10^6 MSC/kg have been reported (Jing et al., 2008; Kang et al., 2012; Koc et al., 2002). The correlations between cell dose and locomotor recovery observed for each administration route in three separate univariate meta-regressions could not be reproduced in our multivariate meta-regression when adjusting for other explanatory variables. However, this finding could be due to lack of accumulated study power and it should be noted that two of the included studies suggest an association between increased MSC dose and locomotor recovery (Li et al., 2010; Pal et al., 2010).

Strengths and limitations of the systematic review

Our systematic review poses a number of advantages compared to previous attempts to summarise the evidence of stem cell therapies for SCI (Tetzlaff et al., 2011). First, our up-to-date literature search yielded

more than 50 additional eligible studies, thus accumulating adequate study power to detect the smallest possible minimal relevant difference in BBB score means (1.0) as witnessed by our TSAs. Second, our search also included the EMBASE bibliographic database, which is known to contain a substantial number of especially non-English journals not registered elsewhere. The actual degree of reference overlap varies according to the medical topic but a comprehensive bibliographic search normally requires the inclusion of EMBASE (Suarez-Almazor et al., 2000). Accordingly, we were able to identify a substantial number of eligible non-English references which hitherto have remained relatively unnoticed. Third, we assessed study risk of bias using quality components based on empirical evidence (Hrobjartsson et al., 2013; Savovic et al., 2012; Wood et al., 2008). Fourth, we addressed the risk of publication bias. Fifth, we contacted investigators in case of inadequate reporting. Sixth, we explored study heterogeneity and sought to identify potential explanatory variables. Finally, we quantified the intervention effect using advanced statistical methodology (meta-analysis, TSA, subgroup analysis, and meta-regression).

Our findings and interpretation are closely related to the experimental design and methodological quality of the included studies. Three major barriers impede successful clinical translation of interventional animal studies, namely low internal validity (systematic error [bias] and random error [play of chance]), publication bias, and low external validity. Although it is always possible to increase internal validity of a study and properly address publication bias, external validity poses unique challenges which are not easily resolved (Akhtar et al., 2008). Accordingly, a number of cases have illustrated that translation from promising animal studies to clinical trials is not straightforward and the usefulness of animal studies as a means of guiding future clinical research has been challenged (Sandercock and Roberts, 2002). For example, in an analysis of 76 highly cited animal studies (median citation count: 889) published in leading scientific high-impact journals, only about one third of the papers were later translated at the level of human randomised clinical trials (Hackam and Redelmeier, 2006).

Internal validity

The methodological quality of interventional animal studies should be no less rigorous than clinical trials since preclinical research often forms the basis on which subsequent early clinical trials are designed and conducted. Internal validity is the sine qua non and refers to how close the intervention effect size in the study sample reflects the true intervention effect size in the population from which the sample was drawn. Risk of bias due to inadequate randomisation (selection bias), non-blinded study personnel (performance bias), or non-blinded outcome assessors (detection bias) is associated with overestimation of intervention effect size in clinical trials, especially for subjective outcomes such as BBB scoring (Hrobjartsson et al., 2013; Savovic et al., 2012). Empirical research suggests that study methods tend to be as good as or better than reported (Hill et al., 2002; Hrobjartsson et al., 2009; Soares et al., 2004), although reporting of unclear allocation concealment may reflect actual study conduct (Pildal et al., 2005). Unfortunately, the internal validity of animal studies is generally poor (Bebarta et al., 2003; Crossley et al., 2008; Vesterinen et al., 2010) and hence inadequate reporting of study methods can impede the assessment of quality and the risk of bias of the results. Limitations in reporting of study methods may therefore have attenuated estimated effects of study design characteristics. For example, in our systematic review, the label 'unclear' was frequently applied because the method was not reported in the reference. However, reported study methodology need not to correspond to how a study was actually conducted, since a well-conducted study may be reported badly (Huwiler-Muntener et al., 2002) and vice versa. In our study we were not able to demonstrate a statistically significant association between methodological components and treatment effect after adjusting with meta-regression. This could be due to lack of adequate meta-analytic study power. Another explanation is that poor reporting of the studies could have led to misclassification of studies, e.g., some studies that did not mention whether or not the assessments were blinded could in fact, have been blinded. Importantly, our subgroup analyses stratified according to methodological components would still seem to suggest a beneficial effect of MSCs even if studies of lower quality were excluded.

An increasing amount of preclinical research and clinical trials is being conducted in less socio-economically developed countries without longstanding tradition in modern biomedical research (Bajpai and Saraya, 2009; Malakoff, 2008; Varawalla, 2007), and stem cell research is no exception to this trend (Cohen and Cohen, 2010; McMahon et al., 2010; Ryan et al., 2010; Salter et al., 2007; Yuan et al., 2012). It has therefore been recommended that researchers undertaking systematic reviews should consider how to manage research data from these countries (Panagiotou et al., 2013; Vickers et al., 1998), since they tend to show significantly more favorable treatment effects for the same intervention compared to studies from more developed countries (Panagiotou et al., 2013). Such discrepancies may reflect known geographical biases in reporting or study design (Vickers et al., 1998; Wu et al., 2009; Zhang et al., 2011) as well as genuine differences in baseline risk or treatment implementation (although the latter would seem unlikely in the case of animal studies). In accordance with the general trend in global stem cell research, a large proportion of the included studies in our systematic review were from less developed countries. However, our subgroup analysis could not detect a statistically significant difference in reported locomotor recovery. This, however, could be due to lack of adequate meta-analytic study power.

Publication bias

Similar to clinical trials, the unintentional exclusion of eligible animal studies in a systematic review will usually result in an overestimation of the intervention effect size simply because unpublished studies often contain neutral or negative data (Sena et al., 2010). Unfortunately, publication bias is prevalent in animal research (Perel et al., 2007). To assess the level of publication bias we generated a funnel plot, but did not detect any publication bias against missing negative studies using two formal testing methods.

External validity

External validity represents the concept of generalizing observations and conclusions from a selected study sample to future populations of interest. Following this context, translation of animal studies to clinical trials can be regarded as an extreme case of external validity. Factors that impede the clinical external validity of animal studies are many (Curt, 2012) including differences in anatomy and pathophysiology (Howells et al., 2010), animal strains (Mills et al., 2001; Popovich et al., 1997; Rex et al., 2007), disease model (Onifer et al., 2007), co-morbidity (Crossley et al., 2008), intervention schemes (Perel et al., 2007), and outcome measure (Dietz and Curt, 2006).

We included only one mammal species to enable the use of an identical, valid and reliable locomotor rating scale and because inflammatory reactions after SCI may differ between experimental animal species (Sroga et al., 2003) thus potentially adding further experimental heterogeneity to the meta-analysis. We focused on the laboratory rat for a number of reasons. First, rats and humans have similar immune cell and cytokine response following SCI, although timings are different (Beck et al., 2010; Fleming et al., 2006; Kwon et al., 2010; Stammers et al., 2012) and differences in the magnitude and duration of macrophage activation throughout the lesion exist between rat strains (Popovich et al., 1997). Second, although induced trauma in rats does not addresses all aspects of spinal damage (Onifer et al., 2007) it fairly reflects the condition in human patients (Metz et al., 2000). Unfortunately, none of our included studies assessed the effect of MSCs on cervical SCI. Third, rat MSCs share similar

characteristics with human MSCs irrespective of strain (Barzilay et al., 2009; Harting et al., 2008). Fourth, the reliability of the BBB scale as assessment for locomotor recovery is well recognised (Basso et al., 1996), and the crucial break point for locomotion is the onset of weight bearing, on which depends proper hind limb stepping (Sedy et al., 2008). Although originally developed for contusion injuries, the scale also works well for other types of injuries such as balloon compression lesion (Sedy et al., 2008). However, it is important to emphasise the non-linear nature of this scale. Thus, e.g. three points increase on the scale could mean a clinically relevant improvement or not, depending where on the scale this is observed. Furthermore, any attempt to translate a BBB difference of 3.9 into a clinically relevant SCI quantitative outcome measure would of course be nonsensical. Finally, it should also be taken into account that the BBB score may be regarded as unreliable as a sole neurobehavioral outcome assessment. The reporting of other neurobehavioral outcome measures was, however, more infrequent thus making quantitative pooling unfeasible for meta-analysis.

Taken together it has been suggested that rats and humans have an analogous relationship with respect to functional, electrophysiological, and morphological outcomes. Furthermore, the techniques for evaluating the extent and severity of SCI in humans and rats are of comparable value indicating that the rat serves as an adequate animal model for research on functional and morphological changes after SCI and the effects of new treatment strategies. Unfortunately, there is no consensus on how locomotor changes in animals should be interpreted both with respect to the potential effect size and specific aspects for human locomotion, such as postural stability, weight bearing, duration and speed (Curt, 2012). Recovery time for rats reaching final stationary plateau phase is considerably shorter compared to humans (Curt, 2012). Notably, many BBB score assessments were conducted at final follow-up before reaching stationary plateau phase, thus likely to underestimate the true average intervention effect size, an observation which our multivariate meta-regression supports. Finally, it is important to stress that none of the included studies assessed the use of MSCs on a cervical SCI model, which complicates the translation of our results further to humans where cervical SCI is frequent.

In the recognition of failing translation of promising animal studies to clinical effect, Kwon et al. have recently proposed a grading system to evaluate the translational potential of experimental neuroprotective therapies for acute SCI (Kwon et al., 2011c). Using this 5-item grading system we evaluated MSCs for SCI. Interestingly, despite the lack of cervical models we estimated a calculated a score substantially above the hitherto highest ranking intervention (minocycline), which is due to high scores of other items rated as highly important by this consensus scale (Kwon et al., 2011c).

Conclusions

Our systematic review with meta-analyses would seem to suggest that MSCs have substantial beneficial effect on locomotor recovery in rat models of traumatic SCI. The results should, however, be interpreted in light of the known limitations in animal experimental design and methodological quality.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nbd.2013.10.014.

Author contributions

Roberto S. Oliveri conceived and designed the systematic review; developed literature search strategy; screened titles and abstracts; full-paper assessed references; extracted data; assessed quality components; calculated summary effect measures; performed meta-analyses, trial sequential analyses, subgroup analyses, univariate meta-regression and funnel plot asymmetry assessments; interpreted data; drafted the first version of the manuscript, and edited and approved the final manuscript. Segun Bello assessed quality components; performed multivariate meta-regression analysis; interpreted data; co-drafted and approved the final manuscript. Fin Biering-Sørensen co-conceived the systematic review; interpreted data; co-drafted and approved the final manuscript.

Competing interests

The authors have no competing interests.

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