Protocol

Stem cell transplantation improve learning and memory recovery in Alzheimer's disease: A systematic review and meta-analysis of preclinical studies

Background

Alzheimer's disease (AD), the most common form of dementia in people over 65 years, is an irreversible progressive neurodegenerative disease, leading to severe incapacity and death. The hallmark neuropathological features of AD include extracellular as well as intracellular deposition of β-amylloid or Abeta (Aβ) protein, the formation of intracellular neurofibrillary tangles (NFT) and widespread neuronal loss, which cause characteristic neuronal deficits in the cerebral cortical and hippocampal areas associated with cognitive decline. According to the latest estimation, it suggests that more than 35 million people worldwide suffer from AD today, with predictions that there could be more 125 million patients with AD by 2050. Unfortunately, the conventional therapeutic methods can only alleviate some clinical symptoms, and no cure or disease-modifying treatments against AD are currently available.

Recently, stem cells transplantation, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and tissue-derived stem cells, such as bone marrow (BM)-and adipose-derived stem cells, has received considerable attention as a potential approach to various diseases, including AD. There is now considerable preclinical literature on the possible benefits of stem cells transplantation against AD. Stem cells transplantation could lead to improve cognitive and memory performances and increased neuronal survival as a result of the decrease in β-amyloid plaques, neurofibrillary tangles, neurodegeneration, and microglia activation in animal models of AD. Moreover, neural stem cells could ameliorate complex behavioral deficits associated with widespread AD pathology via BDNF.[1] In vitro and in vivo, the transplantations of bone marrow-derived mesenchymal stem cells (BM-MSCs) were shown to ameliorate Aβ-induced neurotoxicity and cognitive decline by inhibiting apoptotic cell death and oxidative stress in the hippocampus. It has been also demonstrated that human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) transplantation could rescue the impaired memory function in AD mouse by reduction of apoptosis and modulation of oxidative stress.[2] Human amniotic epithelial cells (HAECs) transplantation could significantly ameliorate spatial memory deficits in transgenic mice, as well as increased acetylcholine levels and the number of hippocampal cholinergic neuritis.

Although stem cells show a strong candidate for treating AD in animal models, however, further studies are needed to determine the appropriate conditions to
improve the therapeutic effects for AD, such as, which type of stem cells and from what source is best to implant, how many are needed, and where the implanted cells need to be transplanted into. Moreover, to inform decisions regarding the design and conduct of subsequent clinical trials, whether the magnitude of integrative and protective effects is large enough to be potentially clinically meaningful, and whether reports of efficacy in animal models are potentially biased in favor of positive results is also need to be investigated. Therefore, we report a systematic review and meta-analysis of data from controlled studies testing the efficacy of stem cells as a treatment in animal models of AD.

**Objective**
(1) to identify all animal experiments describing the efficacy of stem cells based therapies in models of AD,
(2) to systematically review the literature describing the effect of stem cells based therapies on cognitive impairment in animal models of AD,
(3) to perform a meta-analysis using the DerSimonian and Laird random effects model,
(4) to provide empirical evidence of biological factors associated with greater efficacy,
(5) and to provide an assessment for the presence and impact of possible publication bias.

**Search strategy**
Using pre-specified inclusion and exclusion criteria (see below) we identified all publications reporting the efficacy of stem cells in an in vivo animal model of Alzheimer’s disease by searching) three electronic databases (PubMed, EMBASE, and Web of Science; Jan. 1, 2015) using the search strategy “(stem cell OR stem OR haematopoietic OR mesenchymal) AND (Alzheimer’s disease OR dementia OR Senile Dementia),” with various Boolean operators.

Searches of the databases using the search strategy were preformed independently by two individuals. The bibliographies of relevant articles were used to identify further relevant publications. Abstracts will be independently screened by two reviewers to identify those meeting our inclusion criteria (see below), with differences resolved by discussion with a third reviewer.

**Inclusion and Exclusion Criteria**
We included studies where learning and memory functional outcome in a group of animals with AD and treated with allogeneic or autologous stem cells was compared with learning and memory functional outcome in a control group of animals treated with placebo (saline, culture medium or similar vehicle). Labeling or transfection with markers for cellular tracing and imaging (green fluorescent protein, lacZ, bromodeoxyuridine, superparamagnetic iron oxide particles etc.) were included.

We excluded studies using either differentiated stem cells (for example stem
cells that have been differentiated to an endothelial cell) or stem cells engineered to over- or under-express particular genes, or studies using a co-treatment with another therapy or cell type. And co-culture or concomitant injection with other cell types or use of adjuvant products (e.g., matrices, scaffolding, growth factors) were also excluded.

To be included in the meta-analysis, outcomes must be reported as a behavioral outcome, and must report the number of animals in each group, the mean effect size and variance. Disagreements between investigators were resolved by consensus after discussion.

Data extraction

The following items were extracted by two investigators from each included study: reference details (publication year, and name); recipient animal (rat strain, sex); AD model; stem cells (donor species and tissue source); intervention regime (administration route, and number of injections); outcome assessment.

We extracted details of individual study characteristics from each publication, and where a single publication reported more than 1 experiment; these data were extracted and treated as independent experiments. Where neurobehavioral tests were performed serially, we only extracted data for the final time point.

In cases of missing data, we contacted the authors and requested the additional information. If data were expressed only graphically, numerical values were requested from the authors; if a response was not received, digital ruler software was used to estimate numerical values from the graphs. If required data were not presented or obtainable, the study was excluded from analysis.

Methodological quality of studies

We assessed the methodological quality of the included studies using the criteria based on a checklist as previously described with minor modification.[3] The checklist was comprised of 17 items: (1) Research question specified and clear? (2) Outcome measures relevant for AD research? (3) Are the characteristics of study population clear? [Species, Background/generation, Sex (and distribution), and Age] (4) Presence and correct control group? (5) Where the groups similar at baseline (if not randomized think of weight and sex etc)? (6) Is the experiment randomized? (7) Kind of stem cells mentioned? (8) Age when stem cells transplantation started mentioned? (9) Duration of stem cells transplantation clear and specified? (10) Amount of stem cells mentioned? (11) Administration route specified? (12) Methods used for outcome assessment the same in both groups? (13) Drop outs described for each group separately? (14) Blinded outcome assessment? (15) Was the outcome assessment randomized across the groups? (16) Total number of animals included in statistical analyses clear? (17) Age of sacrificing animals mentioned?

The quality of all studies was assessed independently by two reviewers. It should be noted that the quality assessment assesses mainly the reporting quality. Negative judgment does not necessarily indicate that the experiment has been
carried out insufficiently; it indicates inadequate information to assess quality. (Table 3)

**Statistical analysis**

In line with the *Cochrane Handbook for Systematic Reviews of Interventions*, the global estimated effect of stem cells transplantation on cognitive outcome was determined by calculating standardized mean difference (SMD) and 95% confidence intervals (CI) using a random effects model to avoid heterogeneity [4]. Within- and between-study variation or heterogeneity was assessed using Cochran’s $Q$-statistic [5, 6] and the $I^2$ metric, with a significant $Q$-statistic ($P < 0.10$) indicating heterogeneity among studies, $I^2$ values $\leq 50\%$ indicate acceptable heterogeneity among studies [7].

Stratified meta-analysis was used to explore the influence of the potential factors on estimated effect size [8]. Bonferroni correction was used to adjust significance levels for multiple comparisons. [9, 10]. Meta-regression analyses were conducted to evaluated the extent to which study design characteristics explained differences between studies, and the significance level was set at $P <0.05$. [11] And the presence of small effect sizes was investigated using funnel plots and Egger’s tests. For Egger’s tests, a $P$-value of $< 0.10$ was considered to indicate the presence of small effect sizes [5].

All statistical analyses were performed using Review Manager (version 5.3) and Stata software (version 12.1).

**References**


