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Concise Review

Factors Influencing the Efficacy of Umbilical Cord Blood-Derived Cell Therapy for Perinatal Brain Injury

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Abstract

Introduction: We have previously described preclinical literature which supports umbilical cord blood-derived cell (UCBC) therapy as an efficacious treatment for perinatal brain injury. However, efficacy of UCBCs may be influenced by different patient population and intervention characteristics.

Objectives: To systematically review the effects of UCBCs on brain outcomes in animal models of perinatal brain injury across subgroups to better understand the contribution of model type (preterm versus term), brain injury type, UCB cell type, route of administration, timing of intervention, cell dosage, and number of doses.

Methods: A systematic search of MEDLINE and Embase databases was performed to identify studies using UCBC therapy in animal models of perinatal brain injury. Subgroup differences were measured by chi^2 test where possible.

Results: Differential benefits of UCBCs were seen across a number of subgroup analyses including intraventricular hemorrhage (IVH) vs. hypoxia ischemia (HI) model (apoptosis white matter (WM): chi^2 = 4.07; P = .04, neuroinflammation-TNF-α: chi^2 = 5.99; P = .01), UCB-derived mesenchymal stromal cells (MSCs) vs. UCB-derived mononuclear cells (MNCs) (oligodendrocyte WM: chi^2 = 5.01; P = .03, neuroinflammation-TNF-α: chi^2 = 3.93; P = .05, apoptosis grey matter (GM), astrogliosis WM), and intraventricular/intrathecal vs. systemic routes of administration (microglial activation GM: chi^2 = 7.51; P = .02, astrogliosis WM: chi^2 = 12.44; P = .002). We identified a serious risk of bias and overall low certainty of evidence.

Conclusions: Preclinical evidence suggests UCBCs to show greater efficacy in the injury model of IVH compared to HI, the use of UCB-MSCs compared to UCB-MNCs and the use of local administrative routes compared to systemic routes in animal models of perinatal brain injury. Further research is needed to improve certainty of evidence and address knowledge gaps.

Key words: cord blood stem cell transplantation; fetal blood; brain injuries; perinatal care; systematic review.

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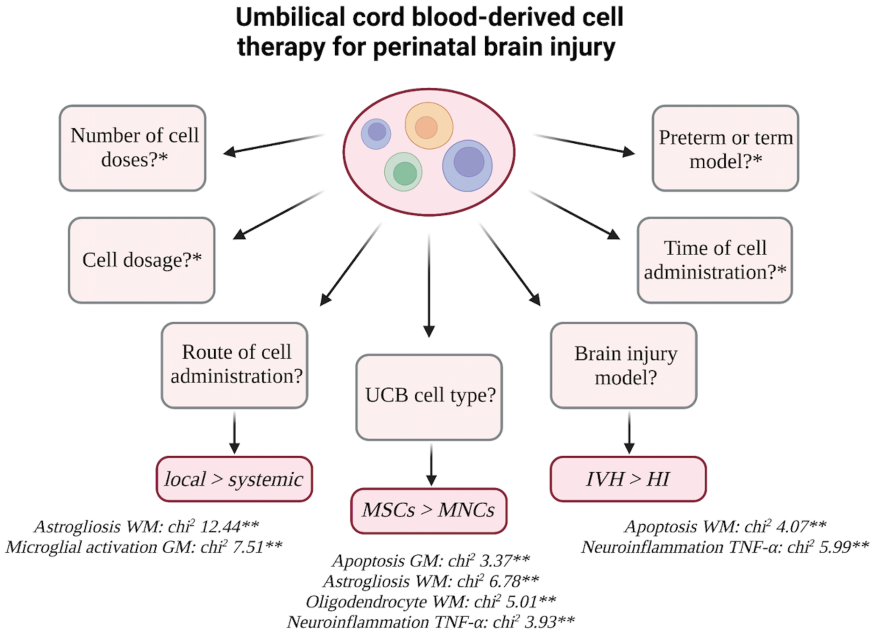
Graphical Abstract



Significance Statement

In neonatal medicine, there is a clear need for the development of new therapies that can provide neuroregenerative benefits for infants with brain injuries. This review offers a unique and comprehensive resource to inform the development of future preclinical and clinical studies. In this review, we systematically reviewed the preclinical literature surrounding UCBCs as a therapy for perinatal brain injury. We investigated the effect variables, such as UCB cell type, timing of administration, and dosage, have on the efficacy of UCB-derived cell therapy in animal models of perinatal brain injury. We identified UCBCs to show greater efficacy in the brain injury model of IVH compared to HI, the use of UCB-derived MSCs compared to MNCs, and the use of local administrative routes compared to systemic routes. In addition to this, we identified knowledge gaps such as the limited preclinical literature surrounding the effect of dose number, sex, and adverse effects.

Introduction

Perinatal brain injury continues to be a major cause of neonatal mortality and life-long neurological disability in both premature and term infants. The term "perinatal brain injury," understood as brain injury occurring during pregnancy or around the time of birth, encompasses a wide range of neuropathologies.[1] These include conditions such as hypoxic ischemic encephalopathy (HIE), intraventricular hemorrhage (IVH), periventricular leukomalacia, and ischemic stroke.[1-3] Perinatal brain injury is common across both developed and low to middle-income nations, with certain forms such as HIE having an incidence as high as 2-4 per 1000 live births.[4,5] Moreover, perinatal brain injuries are significant contributors to the development of a range of serious neurological sequelae including cerebral palsy (CP), which remains the most common physical disability in childhood.[6-8] The high prevalence and morbidity associated with perinatal brain injury highlight the pressing need for developing safe therapies that can effectively reduce and repair brain injuries in infants.

Despite advances in perinatal care which have markedly improved the survival rate of newborns, the available therapies offered for infants born with encephalopathy remains largely supportive.[7] The only neuroprotective option available for term born infants with HIE is therapeutic hypothermia.[9,10] However, this intervention is only shown to reduce neonatal mortality and major morbidity if started within the first 6 h of life for a period of 72 h and deviation from this protocol has shown to worsen neurological recovery.[11] For preterm infants with perinatal brain injury, no current intervention exists except neurosurgical intervention for worsening ventricular dilatation or hydrocephalus following IVH. To see further clinical improvements, new neuroprotective interventions are needed.[9-12] Current preclinical interventions under investigation include creatine, melatonin, erythropoietin, xenon, microRNAs, insulin-like growth factors, and stem cell therapies.[9,13-15] Umbilical cord blood (UCB)-derived cell therapy is one of the most prominent emerging interventions in this area of research and has received a large amount of attention in both preclinical and clinical studies.[16-19]

Previously, we demonstrated UCB-derived cells (UCBCs) were effective in improving both neuropathological and behavioral outcomes in preclinical models.[20] The specific outcomes investigated were apoptosis, astrogliosis, infarct size, microglial activation, oligodendrocyte number, neuroinflammation, and motor function. Importantly, when we applied the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool adapted for preclinical studies, the certainty of this evidence was deemed low.[21] In this review, we aimed to systematically compare the efficacy of UCB-derived cell therapy on brain outcomes across types of perinatal brain injuries, UCB cell types, routes

of intervention, timing of intervention, dosage, and number of cell doses.

Methods

This systematic review and meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and subgroup analysis was performed using a protocol based on The Cochrane Handbook.[22,23] The research protocol was registered on PROSPERO (CRD42022275764).

Selection Criteria

Published preclinical studies of any design investigating UCBC therapy for perinatal brain injury were assessed for eligibility. Inclusion criteria consisted of (1) an animal model of perinatal brain injury, (2) an intervention arm that used any UCB cell or subtype, (3) comparator of no intervention or placebo, and (4) assessed structural or functional brain outcomes. Exclusion criteria included non-perinatal brain injury models, adult animal models, non-UCB-derived cells (ie, derived from umbilical cord tissue), and studies that assessed the efficacy of UCBCs in combination with other interventions. Review articles, conference abstracts, studies where full text was not available, and studies unable to be retrieved in English were excluded.

Search Strategy

MEDLINE and Embase databases were searched via Ovid using a combined search strategy conducted by authors, EP and TN. To ensure recent studies were not missed, the search strategy was conducted on June 24, 2021, April 19, 2022, and additional citation searches was performed in August 2022. The advanced search strategy is presented in Supplemental 1.

Study Selection Process

All studies were exported into Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia, available at www.covidence.org). Duplicates were automatically removed using Covidence in conjunction with manual deduplication (EP, TN). Title and abstract screening and full-text screening were independently performed by 2 reviewers (EP, TN). Disagreements were resolved via discussion with a third reviewer.

Data Extraction

Relevant data were independently extracted by 2 review authors (EP, TN). Data extracted included animal species, type of perinatal brain injury, age of injury induction, control details, and intervention characteristics such as cell type, origin species, route of administration, timing of administration, cell dosage, and the number of cell doses administered. When outcome data was published in a figure without tables or text to ascertain values, PlotDigitizer (version 2.6.9) was used to quantify the data. For papers with missing data, specifically standardized mean difference (SMD), *n* number and standard deviation (SD) or standard error (SE), corresponding authors were contacted a total of 3 times. If authors did not respond, the paper was excluded from the meta-analysis for that particular outcome.

Data Synthesis

Data were synthesized using Review Manager Software for meta-analysis (RevMan, version 5.4). Due to the expected heterogeneity across continuous data measurements, we used a random-effects, inverse variance model to calculate the standardized mean difference (SMD) and 95% CI. The I^2 statistic was used to measure heterogeneity, with 25% considered low, 50% considered moderate, and 75% considered high heterogeneity.[22]

Subgroup Analysis

We aimed to investigate if the intervention effect varied with different patient population and intervention characteristics. Previously, we investigated the brain outcomes of apoptosis, astrogliosis, infarct size, microglial activation, neuron number, oligodendrocyte number, neuroinflammation, and motor function.[20] For each brain outcome, we planned to undertake a subgroup analysis of the following pre-specified variables:

- Model type
- Brain injury type
- UCB cell type
- Timing of cell administration
- Route of cell administration
- Cell dosage
- Number of cell doses

For each subgroup analysis, we considered the criteria of (i) whether a statistically significant subgroup difference was detected, (ii) the covariate distribution, (iii) the plausibility of the treatment effect, (iv) the importance of the treatment effect, and (v) the possibility of confounding.[23] Subgroup differences were measured using the chi^2 test, which tested the difference between the pooled effect estimate (ie, SMD) between subgroups. As recommended by The Cochrane Handbook we planned to not compare within-subgroup statistics such as SMDs.[22] We defined a statistically significant subgroup effect as one where the covariate considered in the subgroup analysis modified the treatment effect by a *P*-value less than 0.1 as recommended by The Cochrane Handbook.[22] The covariate distribution was taken into account by considering the number of studies and study entries included in each subgroup analysis. The plausibility of the treatment effect was evaluated by considering whether evidence currently existed for the observed treatment effect in different studies of similar interventions. The importance of the treatment effect was considered by acknowledging the size of the measured subgroup difference within the context of the review limitations. Finally, the possibility of confounding was also considered.

As advised by The Cochrane Handbook at least 10 study entries were required for the subgroup meta-analysis to be eligible.[22] Additionally, a covariate was defined as a subgroup characteristic that included a minimum of 4 study entries. The covariates included in subgroup analyses are detailed below.

Model Type

Covariates were preterm and term models. Insufficient reporting of preterm and term models was found in studies that used mouse or rat models. Thus, after discussion with review authors, rat preterm was defined as injury induction less than post-natal day (PND) 7 and mouse preterm was defined as injury induction less than PND 9.

Brain Injury Type

All brain injury models were extracted from included studies (chorioamnionitis, excitotoxic brain injury, HI, ischemic

stroke, IVH, meningitis, hyperoxia, and FGR). The brain injury model covariates of HI and IVH included a sufficient number of studies for subgroup analysis.

UCB Cell Type

All UCB cell types were extracted from included studies (EPCs, CD34+ cells, CD34– cells, MNCs, monocytes, MSCs, Tregs, and unrestricted somatic stem cells). The UCB cell types of MNCs and MSCs included enough studies to be included as covariates in subgroup analysis.

Timing of Cell Administration

The times of cell administration post-injury induction extracted were grouped as "less than 24 h", "24-72 h," and "greater than 72 h." The covariates of "less than 24 h" and "24-72 h" included a sufficient number of studies for subgroup analysis. Before commencement of the review, we considered how the timing of "early", "moderate," and "late" administration times in relation to humans varied across animal species. However, lack of published literature outlining how these differences vary across animals resulted in the team deciding upon the above time ranges across all species. Subsequently, caution should be taken when evaluating the results yielded from the timing of cell administration.

Route of Cell Administration

All routes of cell administration were extracted from included studies (arterial, intracerebral, intranasal, intraperitoneal, intrathecal, intratracheal, intraventricular, and intravenous). The routes of arterial, intraperitoneal, intrathecal, intraventricular, and intravenous underwent subgroup analysis. The following routes of cell administration were combined as covariates to allow for comparison between systemic and local routes of delivery; arterial and intravenous (systemic circulation) as well as intraventricular and intrathecal (local).

Cell Dosage

To allow for the comparison of cell dosage between animal models, cell dose amounts were extracted as cells per kilogram (kg). If studies did not report this unit, reported animal weights for the specific aged animal were used to calculate the cell dose amount. Studies were divided into the 3 covariates of "25 million cells per kg," "25-100 million cells per kg," and "greater than 100 million cells per kg." Before commencement of the review, we considered how cell dosage in relation to humans varied across animal species. For example, we considered how a "low dose" varied in a rat when compared to a sheep. However, the lack of published literature investigating these differences resulted in the aforementioned dose ranges being employed across species. Subsequently, caution should be taken when evaluating the results produced from cell dosage subgroup analyses.

Number of Cell Doses

Covariates included single and multiple cell doses. Insufficient studies used a multiple-cell dose regimen to allow for subgroup analysis to be performed.

Quality Assessment

Two reviewers (EP, TN) independently assessed the risk of bias of included studies using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool.[23] Disagreements between reviewers were resolved through discussion with additional authors. Funnel plot analysis in conjunction with Egger's test was performed to assess the presence of publication bias using MedCalc for Windows, v20.115 (MedCalc Software, Ostend, Belgium). The certainty of evidence was assessed using the GRADE tool adapted for preclinical studies.[21]

Results

Search Results

A PRISMA flowchart is presented in Supplemental 2. In summary, 1082 citations were identified. After the process of deduplication, 714 papers underwent title and abstract screening using predefined selection criteria. Seventy-two papers underwent full-text screening. Nineteen of these papers were excluded for incorrect population (n = 9), intervention (n = 9), and study design (n = 1). Two additional studies were identified through manual citation searching. After the screening process, a final number of 55 papers were included in this systematic review.

Characteristics of Included Studies

The characteristics of included studies are summarized in Table 1. Studies included preterm (31%) and term (69%) animal models of rats (65%), mice (16%), sheep (13%), and rabbits (6%). The models of brain injury included HI (74%), IVH (13%), ischemic stroke (2%), chorioamnionitis (3%), meningitis (2%), FGR (2%), hyperoxia (2%), and excitotoxic brain lesions (2%). The route of cell administration included systemic circulation (arterial and intravenous) (37%), intraventricular and intrathecal (27%), intraperitoneal (27%), intranasal (3.5%), intracerebral (3.5%), and intratracheal (2%). The timing of brain injury ranged from in utero to PND14. The timing of UCB-derived cell therapy ranged from 1 h to 7 days post-injury induction. UCB cell types included MNC (60%), MSC (17%), CD34+ (10%), EPC (3%), unrestricted somatic stem cells (3%), and others (Tregs, monocytes, CD34– and CD133+). The cell dosage ranged from 0.5 million cells/kg to 800 million cells/kg. Two out of the 55 studies used multiple cell doses of UCBCs.

Effect of Preterm and Term Models on Efficacy of UCB-Derived Cell Therapy

Four of 8 outcomes that underwent subgroup analysis demonstrated a statistically significant difference in the efficacy of UCB-derived cell therapy between preterm and term models as summarized in Supplemental 3. As shown in Supplemental 4A, microglial activation measured in GM, the test for subgroup differences detected a statistically significant subgroup effect in favor of term models (chi^2 = 3.11, P = .08). Five studies (7 study entries) evaluated preterm models and 11 studies (16 study entries) evaluated term models. Thus, the covariate distribution was not concerning for this subgroup analysis. As shown in Supplemental 4B, astrogliosis in GM and Supplemental 4C, infarct size, statistically significant subgroup differences in favor of term models were also detected. However, in both subgroup analyses the covariate distribution was unevenly distributed as 7 of 9 study entries were associated with one study in the preterm subgroup. Thus, conclusions should not be drawn from these subgroup analyses. In contrast, as shown in Supplemental 4D, oligodendrocyte number in WM, a statistically significant difference between preterm and term models was detected in favor

Table 1. Characteristics of included studies.

Study	Model type	Brain injury type	UCB cell type	Cell administration time post-injury	Route of cell administration	Total cells per dose	Number of cell doses
Ahn (2013)[24]	Preterm	Intraventricular hemorrhage	MSCs	2 days	Intraventricular	1×10^5	1 dose
Ahn (2015)[25]	Preterm	Intraventricular hemorrhage	MSCs	2 days	Intracerebral or intravenous	1×10^5 (intracerebral) or 5×10^5 (intravenous)	1 dose
Ahn (2018)[26]	Term	Meningitis	MSCs	6 h	Intraventricular	1×10^5	1 dose
Ahn (2021)[27]	Preterm	Intraventricular hemorrhage	MSCs	2 days	Intraventricular	1×10^5	1 dose
Aridas (2016)[28]	Term	Hypoxia ischemia	MNCs	12 h after birth	Arterial	1×10^8	1 dose
Baba (2019)[29]	Term	Hypoxia ischemia	MNCs	21 days	Intravenous	5×10^6	1 dose
Bae (2012)[30]	Term	Hypoxia ischemia	MNCs	1 day	Intravenous	1×10^7	1 dose
Chang (2021)[31]	Term	Hypoxia ischemia	CD34+ or CD34- HSCs	12 h	Intracerebral	1×10^5	1 dose
Cho (2020)[32]	Preterm	Hypoxia ischemia	MNCs	7 days	Intraperitoneal	3×10^7	1 dose
Choi (2021)[33]	Preterm	Hypoxia ischemia	MNCs	7 days	Intraperitoneal	3×10^7	1 dose
Dalous (2013)[34]	Preterm	Excitotoxic brain injury	MNCs	1 or 24 h (intraperitoneal), 6 or 24 h (intravenous) after birth	Intraperitoneal or intravenous	10^6, 3×10^6 or 10^7 (intraperitoneal) 10^6 or 10^7 (intravenous)	1 dose
De Paula (2009)[35]	Term	Hypoxia ischemia	MNCs	1 day	Intravenous	1×10^7	1 dose
De Paula (2012)[36]	Term	Hypoxia ischemia	MNCs	1 day	Intravenous	1×10^6, 1×10^7 or 1×10^8	1 dose
Drobyshevsky (2015)[37]	Preterm	Hypoxia ischemia	MNCs	4 h after birth	Intravenous	2.5×10^6 or 5×10^6	1 dose
GeiBler (2011)[38]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal	1×10^7	
Ghaffaripour (2015)[39]	Term	Hypoxia ischemia	MNCs	7 days	Intravenous	2×10^5	1 dose
Grandvuillemin (2017)[40]	Term	Hypoxia ischemia	MNCs or ECFCs	2 days	Intraperitoneal	1×10^7 (MNC) or 5×10^5 (ECFC)	1 dose
Greggio (2014)[41]	Term	Hypoxia ischemia	MNCs	1 day	Arterial	1×10^6 or 1×10^7	1 dose
Hattori (2015)[42]	Term	Hypoxia ischemia	MNCs	6 h	Intraperitoneal	1×10^7	1 dose
Kadam (2015)[43]	Term	Hypoxia ischemia	CD34+ enriched MNCs	2 days	Intraperitoneal	1×10^5	1 dose
Kidani (2016)[44]	Preterm	Hypoxia ischemia	CD133+ cells	1 day	Intraperitoneal	1×10^5	1 dose
Kim (2012)[45]	Term	Hypoxia ischemia	MSCs	6 h	Intraventricular	1×10^5	1 dose
Kim (2016)[46]	Preterm	Hyperoxia	MSCs	PND5	Intratracheal	1×10^5	1 dose
Ko (2018)[47]	Preterm	Intraventricular hemorrhage	MSCs	2 days	Intracerebroventricular	1×10^5	1 dose
Li (2014)[48]	Preterm	Hypoxia ischemia	MNCs or CD34+ cells	7 days	Intravenous	1.5×10^6	1 dose
Li (2016)[49]	Preterm	Hypoxia ischemia	MNCs	12 h or 5 days	Intravenous	5×10^7	1 dose
Li (2017)[50]	Preterm	Hypoxia ischemia	MNCs	12 h	Intravenous	5×10^7	1 dose
Li (2018)[51]	Preterm	Hypoxia ischemia	MNCs	12 h	Intravenous	5×10^7	1 dose
Lyu (2022)[52]	Term	Hypoxia ischemia	MNCs	1 day	Intravenous	1×10^7	1 dose

Table 1. Continued

Study	Model type	Brain injury type	UCB cell type	Cell administration time post-injury	Route of cell administration	Total cells per dose	Number of cell doses
Malhotra (2020)[53]	Preterm	Fetal growth restriction	MNCs	1 h after birth	Intravenous	2.5×10^7	1 dose
McDonald (2018)[54]	Term	Hypoxia ischemia	MNCs, Tregs cells, monocytes, EPCs	1 day	Intraperitoneal	1×10^6 (MNCs) or 2×10^5 (other)	1 dose
Meier (2006)[55]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal	1×10^7	1 dose
Nakanishi (2017)[56]	Term	Hypoxia ischemia	Rat MNCs	3 days	Intraperitoneal	2×10^6	1 dose
Ohshima (2016)[57]	Preterm	Hypoxia ischemia	CD34+ cells	2 days	Intravenous	1×10^5	1 dose
Park (2015)[58]	Term	Hypoxia ischemia	MSCs	6 h	Intraventricular	1×10^5	1 dose
Park (2016)[59]	Preterm	Intraventricular hemorrhage	MSCs	2 or 7 days	Intraventricular	1×10^5	1 dose
Paton (2018)[60]	Preterm	Chorioamnionitis	MNCs	6 h	Intravenous	1×10^8	1 dose
Paton (2019)[61]	Preterm	Chorioamnionitis	MNCs	6 h	Intravenous	1×10^8	1 dose
Penny (2019)[62]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal	1×10^6	1 dose
Penny (2020)[63]	Term	Hypoxia ischemia	MNCs	1 day (1 dose group) or 1, 3, 10 days (3 dose group)	Intranasal or intraperitoneal	1×10^6	1 or 3 doses
Penny (2021)[64]	Term	Hypoxia ischemia	MNCs	1, 3 and 10 days	Intranasal or intraperitoneal	1×10^6	3 doses
Pimentel-Coelho (2010)[65]	Term	Hypoxia ischemia	MNCs	3 h	Intraperitoneal	2×10^6	1 dose
Purohit (2021)[66]	Preterm	Intraventricular hemorrhage	Unrestricted somatic stem cells	18 h	Intraventricular	2×10^6	1 dose
Rosenkranz (2012)[67]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal	1×10^7	1 dose
Rosenkranz (2013)[68]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal	1×10^7	1 dose
Tsuji (2014)[69]	Term	Ischaemic stroke	CD34+ cells	2 days	Intravenous	1×10^5	1 dose
Vinukonda (2019)[70]	Preterm	Intraventricular hemorrhage	Unrestricted somatic stem cells	18 h	Intravenous or intraventricular	1×10^6 (intravenous) or 2×10^6 (intraventricular)	1 dose
Wang (2013)[71]	Term	Hypoxia ischemia	MNCs	1 day	Intraventricular	3×10^6	1 dose
Wang (2014)[72]	Term	Hypoxia ischemia	MNCs	1 day	Intraventricular	3×10^6	1 dose
Wasielewski (2012)[73]	Term	Hypoxia ischemia	MNCs	1 day	Intraperitoneal or intrathecal	1×10^7	1 dose
Xia (2010)[74]	Term	Hypoxia ischemia	MSCs	3 days	Intracerebral	1×10^5	1 dose
Yasuhara (2010)[75]	Term	Hypoxia ischemia	MNCs	7 days	Intravenous	1.5×10^6	1 dose
Yu (2019)[76]	Term	Hypoxia ischemia	MNCs or CD34+ cells	7 days	Intravenous	1×10^6 (MNCs) or 1.5×10^4 (CD34+)	1 dose
Zhang (2019)[77]	Term	Hypoxia ischemia	MNCs	1 day	Intraventricular	1×10^7	1 dose
Zhang (2020)[78]	Term	Hypoxia ischemia	MNCs	1 day	Intraventricular	3×10^6	1 dose

Abbreviations: ECFC, endothelial colony forming cells; EPCs, endothelial progenitor cells; HSCs, hematopoietic stem cells; MNCs, mononuclear cells; MSCs, mesenchymal stromal cells; PND, post-natal day; Tregs, T regulatory cells.

of preterm models (chi² = 14.37, *P* = .0002). Six studies (8 study entries) assessed preterm models and 3 studies (4 study entries) assessed term models. Thus, the covariate distribution was of minimal concern for this analysis. The remaining 4 outcomes did not show statistically significant differences in the efficacy of UCB-derived cell therapy between preterm and term models (Supplemental 4).

Effect of Type of Brain Injury on Efficacy of UCB-Derived Cell Therapy

As summarized in Supplemental 3, 2 of 2 outcomes that underwent subgroup analysis of brain injury type were associated with a statistically significant subgroup difference in the efficacy of UCB-derived cell therapy. As evident in Fig. 1A, apoptosis in WM, a statistically significant difference between HI and IVH injury models was detected in favor of IVH models (chi² = 4.07, *P* = .04). The covariate distribution was not concerning for this analysis as 3 studies (4 study entries) assessed HI and 3 studies (5 study entries) assessed IVH. In a similar fashion, as presented in Fig. 1B, a statistically significant difference in the efficacy of UCB-derived cell therapy on neuroinflammation as measured by TNF-α was found in favor of IVH over HI injury models (chi² = 5.99, *P* = .01). Six studies (7 study entries) assessed HI models and 5 studies (7 study entries) assessed IVH models.

Effect of UCB Cell Type on Efficacy of UCB-Derived Cell Therapy

Four of 8 outcomes demonstrated a statistically significant difference in the efficacy of UCB-derived cell therapy between

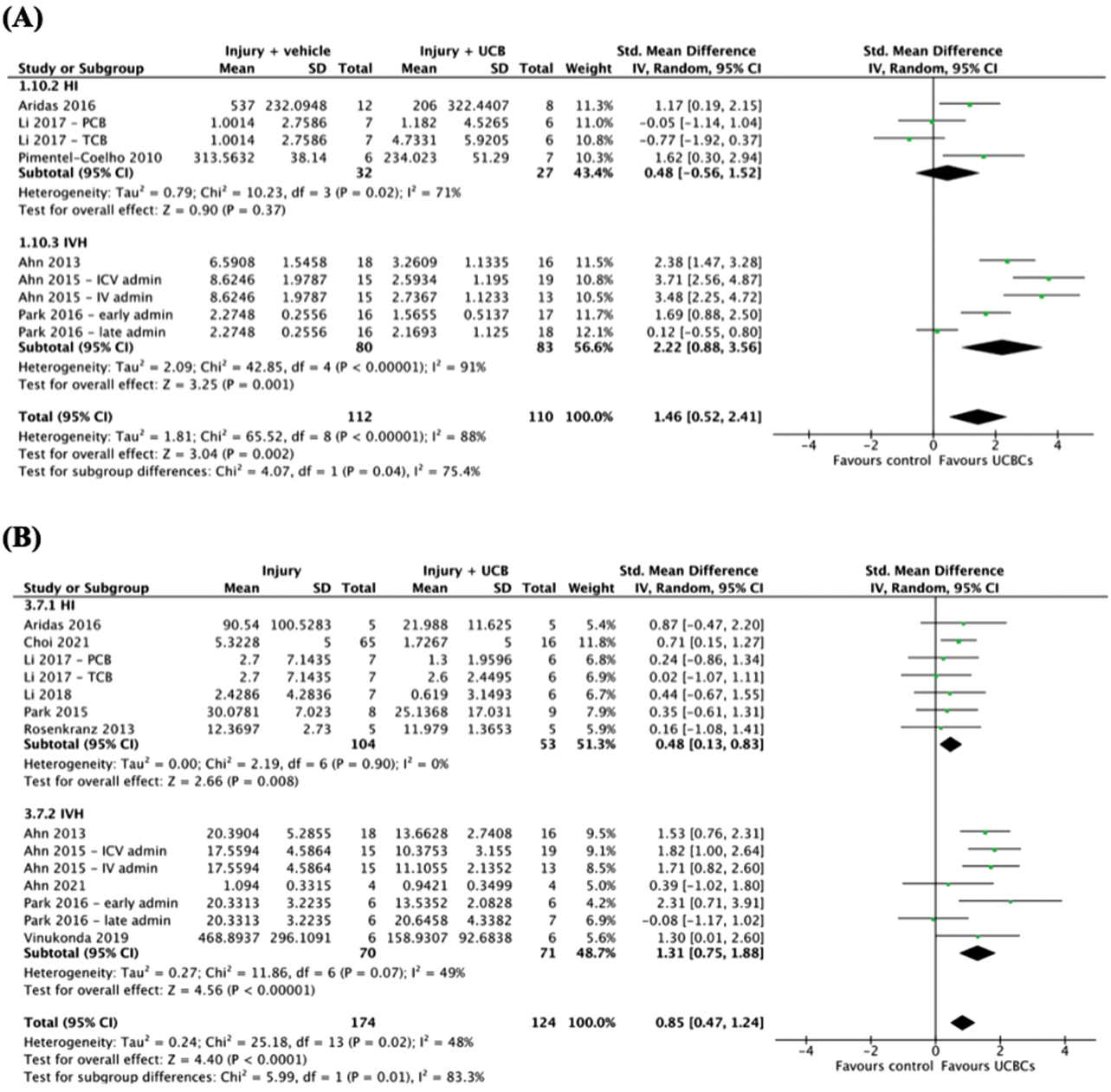


Figure 1. Forest plots demonstrating the effect of brain injury type on brain outcomes of (**A**) apoptosis—white matter; (**B**) neuroinflammation—TNF-α. Abbreviations: admin, administration; ICV, intracerebroventricular; IV, intravenous; PCB, preterm cord blood; TCB, term cord blood.

UCB cell types as detailed in Supplemental 3. As shown in Fig. 2A, a statistically significant subgroup difference between the efficacy of MNCs compared to MSCs when evaluating the outcome of oligodendrocyte number in WM was observed (chi^2 = 5.01, P = .03). This modification of the treatment effect was in favor of MSCs. Five studies (6 study entries) evaluated the efficacy of MNCs and 4 studies (4 study entries) evaluated the efficacy of MSCs. Similarly, as evident in Fig. 2B, neuroinflammation as measured by TNF-α, a statistically significant subgroup difference was detected in favor of MSCs over MNCs (chi^2 = 3.93, P = .05). The covariate was evenly distributed with 6 studies (7 study entries) investigating MNCs and 7 studies (9 study entries) investigating MSCs. Additional subgroup differences were detected in apoptosis in GM (Supplemental 6A) and astrogliosis in WM (Supplemental 6B). Both of these subgroup analyses also detected statistically significant differences in favor of MSCs over MNCs. The remaining 4 outcomes demonstrated no statistically significant differences in the efficacy of UCB-derived cell therapy between MNCs and MSCs (Supplemental 6).

Effect of Timing of Cell Administration on Efficacy of UCB-Derived Cell Therapy

As summarized in Supplemental 3, 4 of 8 outcomes showed a statistically significant difference in the efficacy of UCB-derived cell therapy across different times of cell administration. As shown in Supplemental 7A, apoptosis WM, a

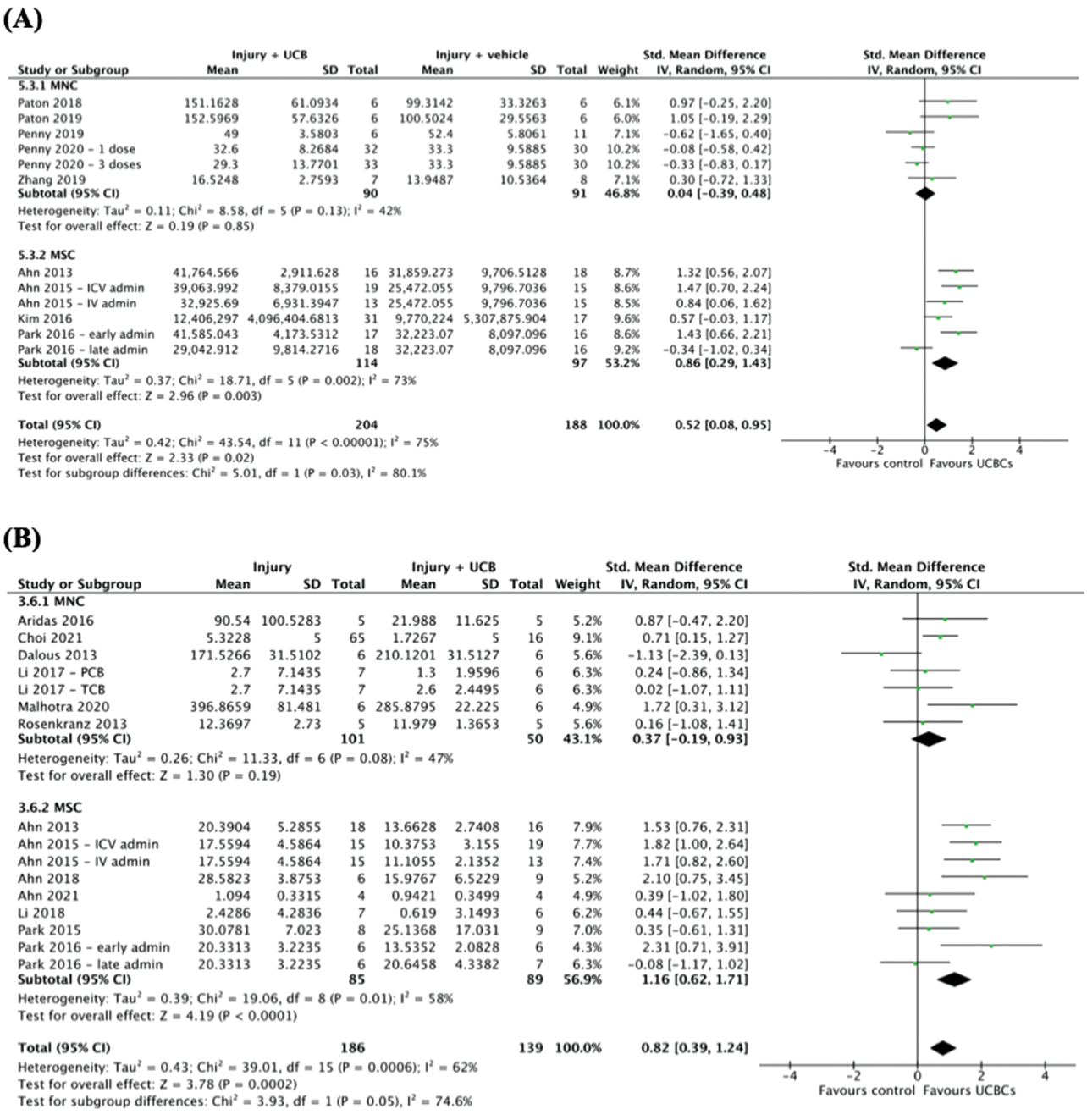


Figure 2. Forest plots demonstrating the effect of UCB cell type on brain outcomes of (**A**) oligodendrocyte number—white matter; **(B)** neuroinflammation—TNF-α. Abbreviations; admin, administration; ICV, intracerebroventricular; IV, intravenous; MNC, mononuclear cell; MSC, mesenchymal stromal cell.

statistically significant modification in treatment effect was seen in favor of cell administration timing of "24-72 h" post-injury induction when compared to "less than 24 h" (chi^2 = 4.72, P = .03). The covariate distribution was of moderate concern for this analysis as 6 studies (7 study entries) formed the "less than 24 h" subgroup and 3 studies (4 study entries) formed the "24-72 h" subgroup. As evident in Supplemental 7B, neuroinflammation as measured by IL-1 β, a statistically significant difference in subgroups of "less than 24 h" and "24-72 h" post-injury induction was also seen in favor of'24-72 h' (chi^2 = 3.31, P = .07). The covariate distribution was not concerning for this subgroup analysis. A similar pattern of UCB-derived cell therapy favoring "24-72 h" post-injury induction over "less than 24 h" was also found in astrogliosis in WM and neuroinflammation as measured by TNF-α. These are presented in Supplemental 7. The remaining 4 outcomes analyzed showed no statistically significant differences in the efficacy of UCB-derived cell therapy between different intervention administration times post-injury induction (Supplemental 7).

Effect of Cell Administration Route on Efficacy of UCB-Derived Cell Therapy

Three of 8 outcomes were shown to have a statistically significant difference in the efficacy of UCB-derived cell therapy across varying routes of cell administration as summarized in Supplemental 3. As seen in Fig. 3A, microglial activation in GM, the test for subgroup differences detected a statistically significant subgroup effect in favor of intraventricular/intrathecal administration over systemic circulation (chi^2 = 7.51, P = .02). A sufficient number of trials were included in the subgroup analysis with 7 studies (10 study entries) contributing to intraperitoneal route of administration, 4 studies (4 study entries) contributing to intraventricular/intrathecal route of administration and 4 studies (6 study entries) contributing to systemic circulation. A similar subgroup effect favoring intraventricular/intrathecal administration over intraperitoneal administration was also detected in astrogliosis in WM (Fig. 3B, chi^2 = 12.44, P = .002). In contrast, as shown in Supplemental 8A, motor function measured by cylinder test, a subgroup difference favoring intraperitoneal route of cell administration over intraventricular/intrathecal route was detected (chi^2 = 6.50, P = .01). The covariate distribution was not concerning for this subgroup analysis as 6 studies (6 study entries) examined intraperitoneal administration and 4 studies (5 study entries) examined intraventricular/intrathecal route of cell administration. The remaining 5 outcomes demonstrated no statistically significant differences in the efficacy of UCB-derived cell therapy between cell administration routes (Supplemental 8).

Effect of Cell Dosage on Efficacy of UCB-Derived Cell Therapy

As summarized in Supplemental 3, 1 of 10 outcomes demonstrated a statistically significant difference in the efficacy of UCB-derived cell therapy with different cell dosages. As shown in Supplemental 9A, apoptosis in WM, a statistically significant subgroup effect was found between "less than 25 million cells per kg" and "25-100 million cells per kg" (chi^2 = 5.63, P = .02). This modification in treatment effect favored "less than 25 million cells per kg." The covariate distribution was not concerning for this analysis as a sufficient number of studies were included in each subgroup. The remaining 9 outcomes which underwent subgroup analysis of dose amount found no statistically significant differences in the efficacy of UCB-derived cell therapy between different doses (Supplemental 9).

Quality Assessment

Quality assessment of the 55 included studies has been previously described.[20] The risk of bias of included studies was assessed using the SYRCLE risk of bias tool and is presented in Supplemental 10.[79] In summary, most biases assessed were judged "unclear" due to lack of sufficient reporting. Additionally, through further assessment via the generation of funnel plots and Egger's test, publication bias was assessed as high across brain outcomes. The certainty of results was assessed using the GRADE tool adapted for preclinical studies.[21] As previously detailed, after the assessment of risk of bias, inconsistency, imprecision, publication bias, indirectness and upgrading, the overall certainty of evidence for our findings was rated as low.

Discussion

Previously we have concluded UCB-derived cell therapy is an efficacious treatment in preclinical models of perinatal brain injury, with benefits seen across both neuropathological and functional outcomes.[20] However, findings were limited by a low certainty of evidence. In this paper, we demonstrated for the first time that variations in study features and design, specifically IVH brain injury, use of UCB-MSCs, and local route (near the site of injury) of administration play a statistically significant role in modifying the treatment effect seen with administration of UCB-derived cell therapy for perinatal brain injury.

Model of Brain Injury

One reason stem cell therapies receive such widespread attention in neonatal research is their unique potential to improve multiple disease states.[80] Despite this, our systematic review identified a heavy focus on HI brain injury, with 41 out of 55 studies investigating HI. Due to the limited studies investigating other forms of brain injury, we were only powered to compare the brain injury models of HI and IVH by subgroup analysis. The outcomes which demonstrated a statistically significant difference between models of brain injury were apoptosis in WM and neuroinflammation measured by TNF-α. The data from our meta-analysis suggests that UCBCs may potentially offer more significant neuropathological improvements in IVH injury when compared to the HI model of brain injury. However, it is important to emphasize this insight is heavily limited by the overall low certainty of our evidence. In addition, high heterogeneity across studies also introduces the possibility of confounding. Nonetheless, our paper highlights the need for further studies to investigate the potential of UCBCs as therapy for brain injury models other than HI. Of the 7 studies we identified that assessed the effect of UCBCs on IVH, none were performed in large animal models. Furthermore, there is no current preclinical literature which directly compares the efficacy of UCB-derived cell therapy across different models of brain injury. Thus, further investigation comparing perinatal brain injuries is warranted and may offer insights into the underlying mechanisms of UCBCs as well as provide essential evidence-based data to inform the development of future clinical trials.

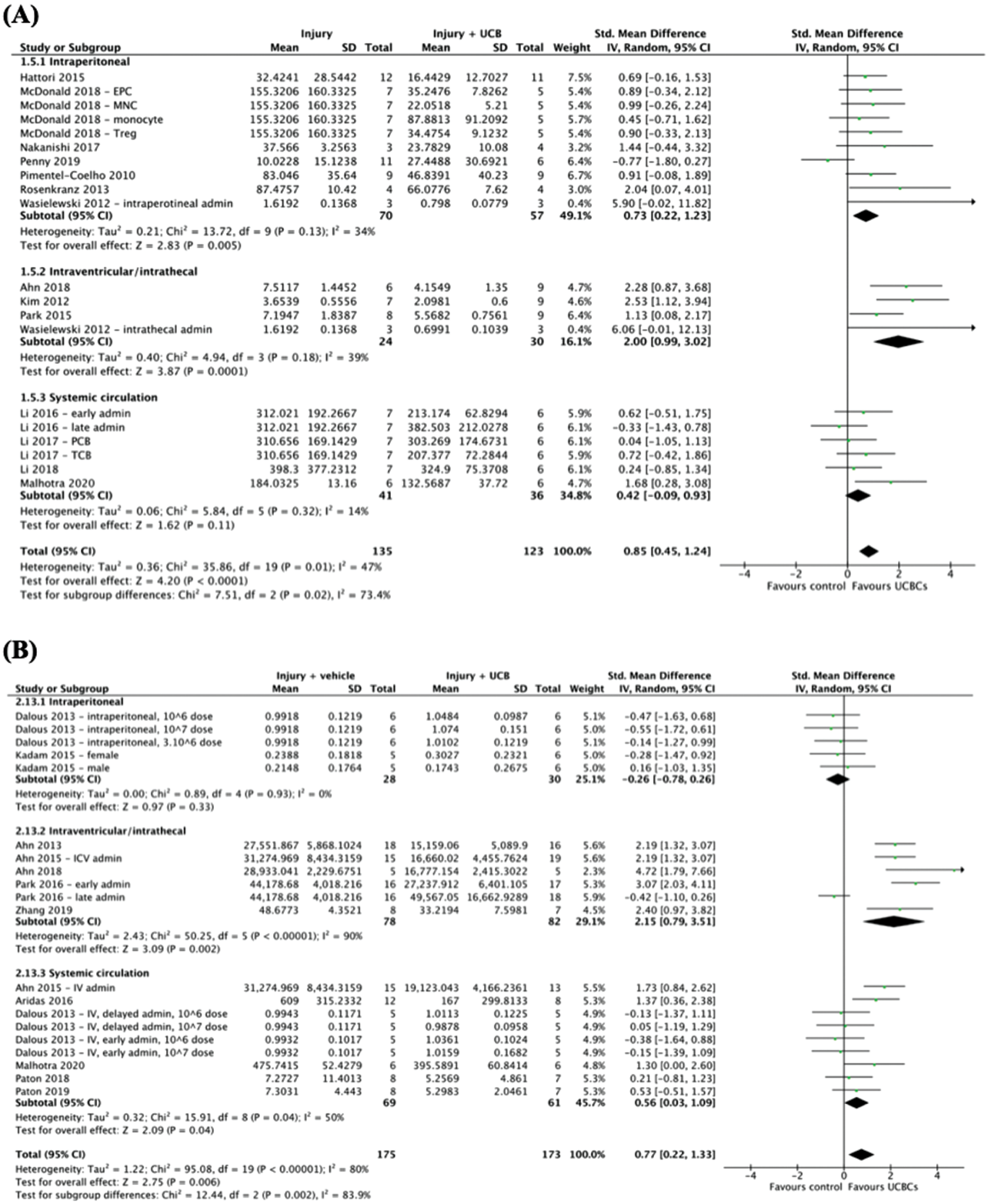


Figure 3. Forest plot demonstrating the effect of route of cell administration on brain outcomes (**A**) microglial activation—grey matter; (**B**) astrogliosis—white matter. Abbreviations: admin, administration; EPC, endothelial progenitor cell; ICV, intracerebroventricular; IV, intravenous; MNC, mononuclear cell; PCB, preterm cord blood; TCB, term cord blood; Treg, T regulatory cell.

UCB Cell Type

UCB refers to blood within the umbilical cord and blood vessels surrounding the fetal component of the placenta.[17] Numerous cell types comprise UCB including HSCs, MSCs, Tregs, monocytes, and EPCs.[81] In our review, all 4 outcomes which demonstrated statistically significant differences between UCB cell types were associated with a favored modification of treatment effect in MSCs over MNCs. Our review provides further neuropathological support for UCB-MSCs as a potential therapeutic option for infants with perinatal

brain injury.[13,61,82] To the best of our knowledge, in the current literature no study is yet to directly compare UCB-MNCs to UCB-MSCs. However, Paton et al. (2019) has investigated UCB-MNCs cells to UC-MSCs and found the cell types had differential effects on WM in the preterm brain.[61] The results of our review are consistent with the reasoning for this differential effect being that MSCs comprise <0.1% of the total MNCs in UCB.[61] The beneficial effects seen in our review are also consistent with a recent systematic review performed by Lehnerer et al. (2022) which found that administration of MSCs (sourced from bone marrow, UCB, placenta, Wharton's jelly, and adipose tissue) significantly favored sensorimotor and cognitive performance in perinatal arterial ischaemic stroke injured animals.[82] Despite our review findings supporting the use of UCB-MSCs over UCB-MNCs, particularly in the context of WM microstructure, it is important to highlight other UCB cell types, such as Tregs and monocytes, did not have sufficient studies to be included in subgroup analysis. Moreover, it is essential to understand our review findings in the context of our quality assessment, which found that the overall certainty of our results was low, primarily due to the high heterogeneity between studies.

Route of Cell Administration

A range of UCB-derived cell therapy delivery routes has been investigated in preclinical literature. UCBCs can be delivered locally around the site of injury (intracerebral, intraventricular, intrathecal, and intranasal) or systemically (intravenous, intraarterial, and intraperitoneal).[7] In our review, 3 outcomes showed statistically significant differences between the method of delivery. Two of these outcomes (astrogliosis in WM and microglial activation in GM) favored intraventricular/intrathecal administration over systemic routes and the 3rd outcome (motor function measured by cylinder test) favored intraperitoneal route of cell administration over local routes. These data are suggestive that UCB-derived cell therapy may potentially be more effective on neuropathological outcomes when UCBCs are administered locally to the injured site where they have been shown to have effects via cell-to-cell contact in addition to paracrine mechanisms.[9,82,83] In the preclinical space, only 3 studies have directly compared the routes of intracerebral, intraventricular, or intrathecal administration to another route of cell administration. Wasielewski et al. (2012) compared the routes of intrathecal to intraperitoneal, Ahn et al. (2015) compared intracerebral to intravenous, and Vinukonda et al. (2019) compared intraventricular to intravenous routes.[25,70,73] Further studies comparing intraventricular or intracerebral routes of delivery to other less invasive local routes such as intranasal delivery and systemic routes would be valuable additions to the current preclinical literature. Additionally, in the clinical setting, the majority of trials have implemented intravenous routes of administration.[16] To the best of our knowledge, there has been one phase one trial using intraventricular transplantation and this was shown to be safe and feasible in extremely premature infants with severe IVH.[84] In comparing this trial to clinical trials performed in children with cerebral palsy, intrathecal, and intraventricular delivery of stem cells have also been shown to have no inferior safety profile to systemic routes in early phase trials.[85,86] Thus, further research is needed to be done to investigating the safety profile, feasibility, and efficacy of local administration routes of UCBCs.

Limitations

We acknowledge there are limitations to this review. Of most importance is the high heterogeneity within the studies investigated. Included studies varied across animal species, brain injury models, UCB cell types, administration routes, cell dosage, measurement tools, and animal sex. Although such heterogeneity enabled subgroup analyses to be performed, the substantial heterogeneity significantly reduced the overall certainty and validity of the evidence. For example, across different animal species an early cell administration time point and high cell dose amount in relation to humans varies considerably. Subsequently, caution should be taken when evaluating the results yielded from timing of cell administration and cell dosage. Additionally, the significant heterogeneity between studies also introduced the possibility for interactions between variables to occur. For instance, when comparing MSCs to MNCs, the dose range, animal species, and injury type varies across studies and thus the possibility of confounding variables is a significant limitation. However, due to the limited number of studies within each subgroup analysis, the ability to explore such interactions was not feasible in this review. Additionally, despite our best efforts to retrieve missing data from respective authors, a number of studies were excluded from respective meta-analyses due to missing data. Thus, as previously discussed, through GRADE analysis the overall certainty of evidence is considered low due to factors such as heterogeneity and serious risk of bias seen across studies.[20]

Furthermore, our review included distinct treatment groups of the same study as individual study entries. Although this method has been implemented across several past reviews, when a limited number of study entries exist within a subgroup, the effect seen in one particular study can substantially influence the overall SMD seen for that subgroup.[22] Similarly, when evaluating the results of this review caution should be taken when subgroups included a limited number of studies. Important to note is 26 of the 44 subgroup analyses performed found no statistically significant differences. To determine if there is indeed a lack of significant differences in these factors or if the review was limited by insufficient power, future meta-analyses incorporating a larger number of studies and homogeneity between studies are needed. In addition to this, the size of subgroup differences detected should also be noted. Fifteen of the 18 subgroup analyses which detected a statistically significant subgroup difference were measured as a *P* value between .001 and .1. By incorporating a larger number of studies and minimizing heterogeneity across studies, our results and findings may have altered.

Another limitation was that both preterm and term injury models were combined in this review. A differential effect was seen in the subgroup analyses which compared preterm and term models. Microglial activation in grey matter demonstrated a statistically significant difference in favor of term models while oligodendrocyte number measured in white matter demonstrated a statistically significant difference in favor of preterm models. One explanation for this observation is that white matter injury is the most common type of brain injury seen in preterm infants.[49] Subsequently, combining both preterm and term models in subgroup analyses such as timing of administration and cell type, is a significant limitation of this review. Furthermore, subgroup analyses of cell dose number, sex, and adverse effects were not evaluated in this review. Recent literature has shown administration of

multiple cell doses is an important factor in the efficacy of UCBCs.[63] However, due to the limited number of studies which implemented a multiple-dose regimen, we were not powered to undertake a subgroup analysis. Similarly, we found most studies in the review did not comment on the safety profile or potential adverse effects of UCBCs. Investigating the safety profile of UCBCs is essential for further progression in clinical research and thus is recommended to be a focus of future preclinical studies.

Additionally, a major limitation of this review was that functional outcomes were restricted to motor function as measured by rotarod and cylinder tests. Other clinically important functional outcomes such as cognitive function were unable to be investigated due to lack of preclinical literature investigating such outcomes and wide variation in measurement tools used across studies. This is a significant limitation as statistical differences in neuropathological biomarkers identified may not correlate with corresponding differences in functional outcomes. Further preclinical research into functional outcomes in addition to standardization of how such outcomes are measured is recommended. This will enable future meta-analyses of functional outcomes to be performed and thus allow for more robust preclinical evidence to inform future clinical research. Finally, in this review we limited our focus to UCBCs. It is important to note there are other sources of cells that have shown potentially neuroregenerative effects such as cells derived from umbilical cord tissue, bone marrow, amnion, and placental tissue.[8,12]

Future Directions

With the increasing number of clinical trials showing beneficial results, the use of UCB-derived cell therapy in the treatment of infants with brain injuries is an exciting possibility. However, this review has demonstrated that further preclinical research is warranted to progress UCB-derived cell therapy along the research pipeline. We recommend continued research of UCB-derived cell therapy in the context of preterm versus term models, physical sex, brain injury models other than HI such as IVH, cell types other than MNCs, timing of administration particularly greater than 72 h post-injury, effect of local routes of administration such as intranasal compared to other local and systemic routes, the effect of cell dosage, the use of multiple cell doses. Additionally, research into functional outcomes and potential adverse effects of UCBCs should be further investigated and performed in large animal models where feasible. To improve the quality of preclinical evidence, we recommend future studies to pre-register study protocol, adopt standardized tests for measuring functional outcomes, report methodology in greater detail such as use of blinding, randomisation, animal sex, survival rate, dosage in cell/kg, sample numbers, and specify error bars as SD or SEM. In addition to this, future research should investigate how across species we define a preterm or term model, low to high cell dosages, and early to late timing of interventions. Forming standardized definitions of such characteristics across animal species will greatly improve the power of future systematic reviews and yield further needed evidence. In summary, further preclinical research into UCB-derived cell therapy for perinatal brain injury is needed to determine and confirm optimal cell type, timing of administration, route of administration, cell dosage, and dose number across varying brain injuries.

Conclusions

This systematic review and meta-analysis of 55 preclinical studies identified UCBCs to show greater efficacy in the brain injury model of IVH compared to HI, the use of UCB-derived MSCs compared to MNCs, and the use of local administrative routes compared to systemic routes. Additional preclinical research, particularly in large animal models, is required so that we can further identify and confirm differences in the efficacy of UCB-derived cell therapy across all investigated variables in addition to dose number, sex, and adverse effects. Research in such areas is crucial to aid in the translation of UCB-derived cell therapy to the clinical setting.

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Conflict of Interest

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Author Contributions

E.P. and T.N.: conception and design, literature searching, collection and/or assembly of data, data analysis and interpretation, risk of bias assessment, manuscript writing. M.S.: conception and design, data analysis and interpretation, risk of bias assessment, manuscript editing. T.P., M.P., L.Z., G.J and S.M.: conception and design, manuscript editing. C.M. and A.M.: conception and design, literature searching, data analysis and interpretation, risk of bias assessment, manuscript editing, supervision.

Data Availability

All datasets and analyses created in this review are available from the authors upon reasonable request.

Supplementary Material

Supplementary material is available at *Stem Cells Translational Medicine* online.

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