



Cell-Based therapy for traumatic brain injury

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Abstract

Traumatic brain injury is a major economic burden to hospitals in terms of emergency department visits, hospitalizations, and utilization of intensive care units. Current guidelines for the management of severe traumatic brain injuries are primarily supportive, with an emphasis on surveillance (i.e. intracranial pressure) and preventive measures to reduce morbidity and mortality. There are no direct effective therapies available. Over the last fifteen years, pre-clinical studies in regenerative medicine utilizing cell-based therapy have generated enthusiasm as a possible treatment option for traumatic brain injury. In these studies, stem cells and progenitor cells were shown to migrate into the injured brain and proliferate, exerting protective effects through possible cell replacement, gene and protein transfer, and release of anti-inflammatory and growth factors. In this work, we reviewed the pathophysiological mechanisms of traumatic brain injury, the biological rationale for using stem cells and progenitor cells, and the results of clinical trials using cell-based therapy for traumatic brain injury. Although the benefits of cell-based therapy have been clearly demonstrated in pre-clinical studies, some questions remain regarding the biological mechanisms of repair and safety, dose, route and timing of cell delivery, which ultimately will determine its optimal clinical use.

Key words: cell-based therapy; stem cells; traumatic brain injury

Editor's key points

- The authors review the mechanisms of traumatic brain injury and the potential place for the use of cell-based therapies.
- They conclude that there is a clear potential for benefit, but substantial work remains in optimising cell-based therapy.

In the United States between 2001 and 2010, severe traumatic brain injury (TBI) was responsible for up to 2 200 000 emergency department visits, 300 000 hospitalizations and 55 000 deaths each year. Traumatic brain injuries contributed to 30% of all

injury-related deaths in the USA. Their economic burden, including direct medical and indirect costs, was estimated in 2010 to be approximately \$76 billion dollars.¹ In 2007, the Brain Trauma Foundation and the American Association of Neurological Surgeons published the third edition of evidence-based guidelines for the management of severe TBI.²

However, because of the severe morbidity and mortality associated with TBI, innovative therapies are needed. Based on promising pre-clinical studies and a few completed clinical trials, cell-based therapy may be such a new, innovative, therapeutic approach. In this review, we describe the pathophysiology of TBI and give a comprehensive overview of the pre-clinical studies on the use of cell-based therapy for TBI. We present the different

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cell types used for treatment, their main biological basis of action, the various animal models utilized, and outline the main results. We also discuss the few published and ongoing clinical trials. This review was created by searching PubMed for relevant studies, considering the following MeSH terms: stem cell, cell-based therapy, and traumatic brain injury, from the first published studies on this topic in 2000 until 2014, and examining the clinicaltrials.gov database and major published reviews. Of 896 articles initially selected, we eliminated articles not directly focused on brain trauma or cell-based therapy and finally reviewed 89 articles, among which, 68 were pre-clinical studies.

Pathophysiology of traumatic brain injury

Time dependent injury, neuronal loss and the inflammatory micro-environment

TBI can result from direct impact or from extreme acceleration-deceleration and rotational forces. The injury evolves over two phases. The primary phase corresponds to immediate damage to the central nervous system with massive depolarization of brain cellular components, resulting in a major release of inflammatory neurotransmitters, inducing monocyte/macrophage-mediated phagocytosis and complement-mediated cytolysis, and diffuse neuronal dysfunction.³ Initial forces can also disrupt the blood brain barrier, further aggravated by early expression of high concentrations of glucose transporter-1 and synthesis and release of nitric oxide.⁴ Consequently, the resulting cerebral haemorrhage and oedema can increase intracranial pressure and lead to cerebral ischaemia. The secondary phase starts a few hours after the injury and can last several days. It is mostly characterized by an intracellular influx of calcium, free radical generation with lipid peroxidation, and mitochondrial dysfunction,⁴ leading to apoptosis and necrosis of neuronal cells.

The neuronal loss after TBI is both focal and diffuse as a consequence of the primary and secondary phases of the injury. The hippocampus is especially vulnerable to the neuronal loss, even in the absence of elevated intracranial pressure,⁵ explaining why many studies have been interested in this cerebral region. Apoptotic neurones have been observed in the hippocampus even up to 12 months after TBI, correlating with memory impairment both in animal models and humans.⁶ TBI is responsible for an acute inflammatory environment, with monocyte/macrophage-mediated phagocytosis and complement-mediated cytolysis, which can persist several weeks after the injury.⁷ Although TBI can up-regulate neuronal growth factor (NGF) and brain derived neurotrophic factor (BDNF) and down-regulate neurotrophin-3, this inflammatory environment may impede the function of endogenous stem cells in repair.⁸

Neurogenesis and angiogenesis

Neurogenesis and angiogenesis are stimulated after TBI. After a short proliferation phase, neural stem cells (NSC) migrate from the sub-ventricular zone (SVZ) to the site of injury and differentiate into neuronal and glial cells, stimulated by growth factors released by endothelial cells (Fig. 1). In animal models, the ipsilateral SVZ proliferation increases two to four-fold after TBI, while contralateral SVZ proliferation increases to a lesser extent.⁹ Also, active angiogenesis has been observed three days after an ischaemic insult. Nevertheless, even if neuroblasts have been shown to migrate to areas of injury, their ability to replace neuronal loss is uncertain.⁹ Furthermore, the reparative mechanisms are often overwhelmed by the resulting inflammatory

neurotransmitters, cerebral haemorrhage and oedema after TBI. Multiple investigators have studied the effect of various stem and progenitor cells as therapy in this injury environment, to minimize the severity of TBI.

Pre-clinical studies using stem and progenitor cells as treatment for traumatic brain injury

Reported mechanisms of action

Various cell types have been used as potential therapy for TBI: mesenchymal stem cells (MSC), NSCs, neural progenitor cells (NPC), NTera2 (NT2) cells, embryonic stem cells, multipotent adult progenitor cells, and endothelial progenitor cells (Supplementary Table). Currently, several different mechanisms of action have been postulated to explain the therapeutic effects of transplanted stem and progenitor cells delivered after TBI (Fig. 2). The promotion of cell replacement by the differentiation of NSCs and MSCs was first hypothesized to be an essential mechanism of action of stem and progenitor cells after TBI.^{10–11} But our current knowledge suggests that improvements after TBI may essentially result from paracrine and systemic effects, via the secretion of chemokine and growth factors,^{12–14} decreasing oedema and inflammation caused by TBI, and enhancing endogenous neurogenesis, angiogenesis and vasculogenesis.¹⁵ Stem and progenitor cells may also stabilize damage cells via gene and protein transfer, by inter-cellular contact or fusion,¹⁶ and may develop pathways between the SVZ and the site of injury by a 'biobridge,' enhancing the migration of host neurogenic cells.¹⁷

Mesenchymal stem cells

Cells origin, dose, and potency

MSCs were the most frequently used stem cells for therapy in experimental TBI (Supplementary Table). Previously, cell-based therapy with MSCs was shown to be safe clinically, when administered in patients with various acute organ injury such as myocardial infarction, acute kidney injury, stroke, etc.¹⁸ For pre-clinical studies in TBI, MSCs were mainly isolated from rat and human bone marrow,^{19–20} but were also isolated from human umbilical cord,²¹ rat and human adipose tissue,^{22–23} and human amniotic membrane.²⁴ No study compared the effects of MSCs on TBI according to their site of isolation (i.e. bone marrow, adipose tissue, placenta). The primary mechanism of action proposed initially was the ability of MSCs to differentiate into neural cells, but there is little evidence that these cells can transform into functional neurones.^{25–26} Most mechanistic studies now deal with the ability of MSC to secrete paracrine soluble factors, which stabilize the endothelium preventing excessive permeability and suppress cells of the innate and adaptive immune system.

The administration dose of MSCs used in experimental TBI models in rodents varied from 1.5×10^5 to 2×10^7 cells per kg body weight, with the average dose being mostly between 10^6 and 10^7 . Lower doses were reserved for stereotactic injection,^{14–17–21–24–27} or internal carotid artery,²⁹ or lateral ventricle^{30–31} delivery. In studies with i.v. administration, higher cell dose was associated with higher cell survival rate, but without better functional improvement.^{32–33}

Transplanted MSCs were cultured without growth factors in the large majority of pre-clinical studies. However, some groups cultured MSCs with NGF and BDNF^{23–34–35} or epidermal growth factor (EGF) and fibroblast growth factor (FGF)-2.^{21–24} NGF and BDNF increased the survival rate and the microtubule-associated

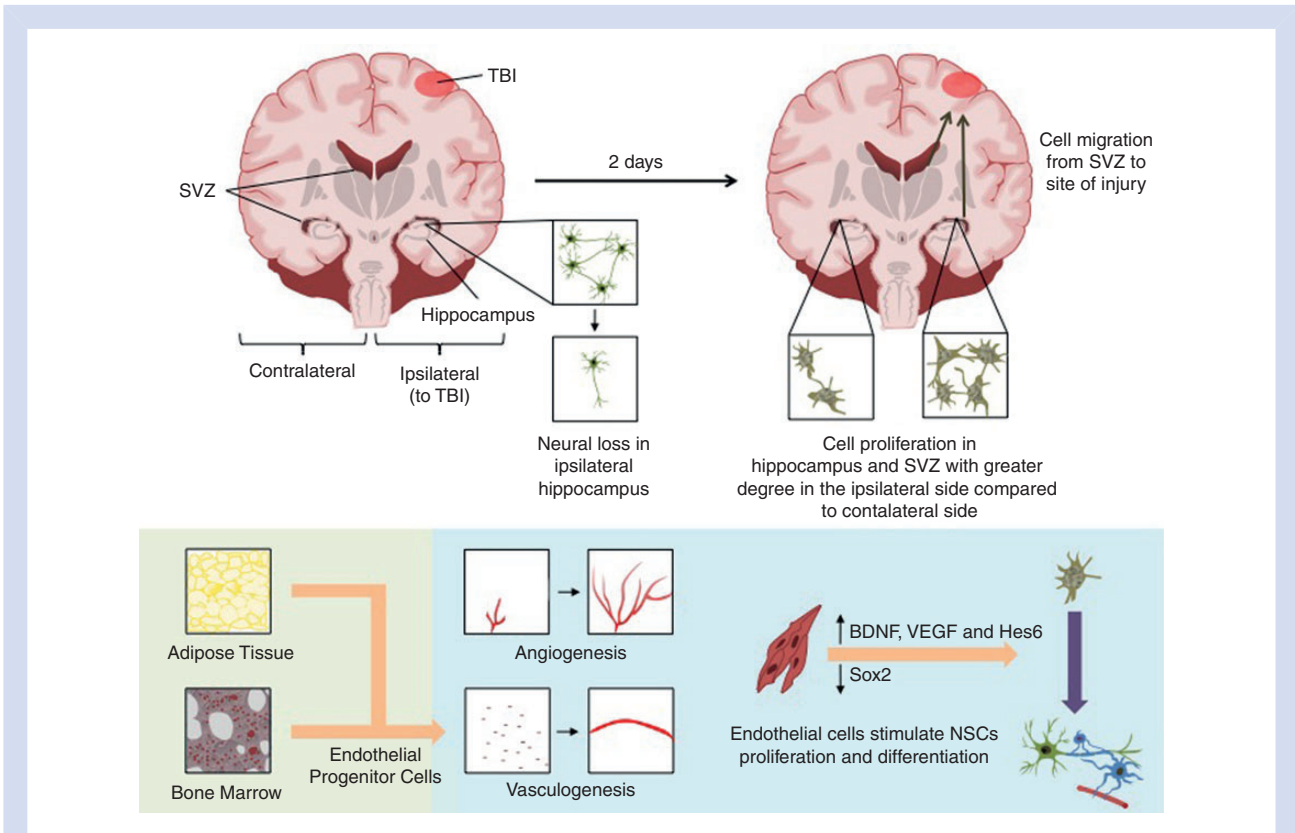


Fig 1 Neurogenesis and Angiogenesis After Traumatic Brain Injury. Neurogenesis occurs throughout adult human life in two different zones of the brain: the subgranular zone of the hippocampus dentate gyrus, and the subventricular zone (SVZ) of the lateral ventricles - olfactory bulb pathway. The hippocampus and the SVZ generate neural stem cells (NSCs), which are self-renewing and multipotent. NSCs can generate neural progenitor cells (NPCs), which do not maintain the NSCs pool but rather generate neuronal and glial cells. After the initial neuronal loss in the hippocampus after traumatic brain injury (TBI): (A) Neurogenesis increase in the SVZ and in the hippocampus as early as two days, essentially in the ipsilateral side of the injured brain but also in the contralateral side to a lesser extent. Neurogenesis remains usually high for two weeks in the SVZ and one month in the hippocampus, and sometimes last up to one year. NSCs can therefore migrate directly from the SVZ to the site of injury and differentiate into neuronal and glial cells. New neurones can extend axonal projections to the cornu ammonis (CA)-3 region of the hippocampus two weeks after TBI, leading to cognitive improvement. (B) Angiogenesis from pre-existing vessels and vasculogenesis from bone-marrow and adipose-tissue derived endothelial cells are also stimulated after TBI. Increased angiogenesis has been shown three days after injury, with proliferation of endothelial cells by twelve hours. Endothelial cells also stimulate the proliferation and differentiation of NSCs and migration of neuroblasts, in part through the production of soluble growth factors such as the brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). Endothelial cells also promote neuronal differentiation by up- and down- regulating Hes6 and Sox2 expression, respectively. Although effective after minor brain injury, these mechanisms may be insufficient after severe TBI. BDNF, brain derived neurotrophic factor; CA, cornu ammonis; Hes6 and Sox2, transcription factors; NPC, neural progenitor cells; NSC, neural stem cells; SVZ, subventricular zone; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor.

protein-2 expression of transplanted MSCs,^{34 35} whereas EGF and FGF-2 did not change the expression of these neurone specific genes.^{21 24} Nevertheless, by delivering FGF-2 with bone marrow derived MSCs by stereotactic injection, Bhang and colleagues²⁸ found increased expression of neuronal and astrocytic markers and improvement in animal motor function. Liu and colleagues³⁶ directly injected FGF-2 into the lateral ventricle and showed a higher survival rate in transplanted bone marrow derived MSCs and increased differentiation into neuronal and glial cells, but no functional recovery.

In other studies, MSCs were transduced or transfected to enhance their survival in hypothermia, increase insulin production or endothelial and neuronal growth.^{27 37-39} MSCs, transduced with the temperature sensitive antigen tsA58 SV40LT, displayed higher survival rates and proliferation in the context of hypothermia.³⁸ Enhanced concentrations of BDNF were found in cerebrospinal fluid after animal treatment with bone marrow derived MSCs transfected with the BDNF gene.³⁷ Hippocampal cell loss was reduced with bone marrow derived MSCs, transfected with

glucagon like peptide-1.²⁷ And the use of MSCs, transfected with anti-tissue inhibitor of matrix metalloproteinase-3, restored adherent junctions in the injured brain through increase in VEGF-A signalling.³⁹

Small animal models

Pre-clinical studies were performed primarily in Sprague-Dawley and Wistar rats, with a few using C57BL/6 mice.^{14 30} Cyclosporine A was administered as an immune suppressive in some studies with animals receiving human MSCs,^{28 30 31} although Pischiutta and colleagues³¹ found no clinical or biological benefit of Cyclosporine A in mice receiving human MSCs. The principal model of TBI used was the controlled cortical impact (CCI),^{17 19 20 22-25 27 30-35 39-49} considered to induce mostly focal injuries. The second model most widely used was the fluid percussion injury (FPI),^{37 38 50 51} inducing mostly diffuse injuries. Weight drop impact (WDI)^{21 29 36} and the penetrating brain injury (PBI),¹⁴ considered to induce respectively diffuse and focal brain damages, and the cryogenic lesion²⁸ were other models used as well.

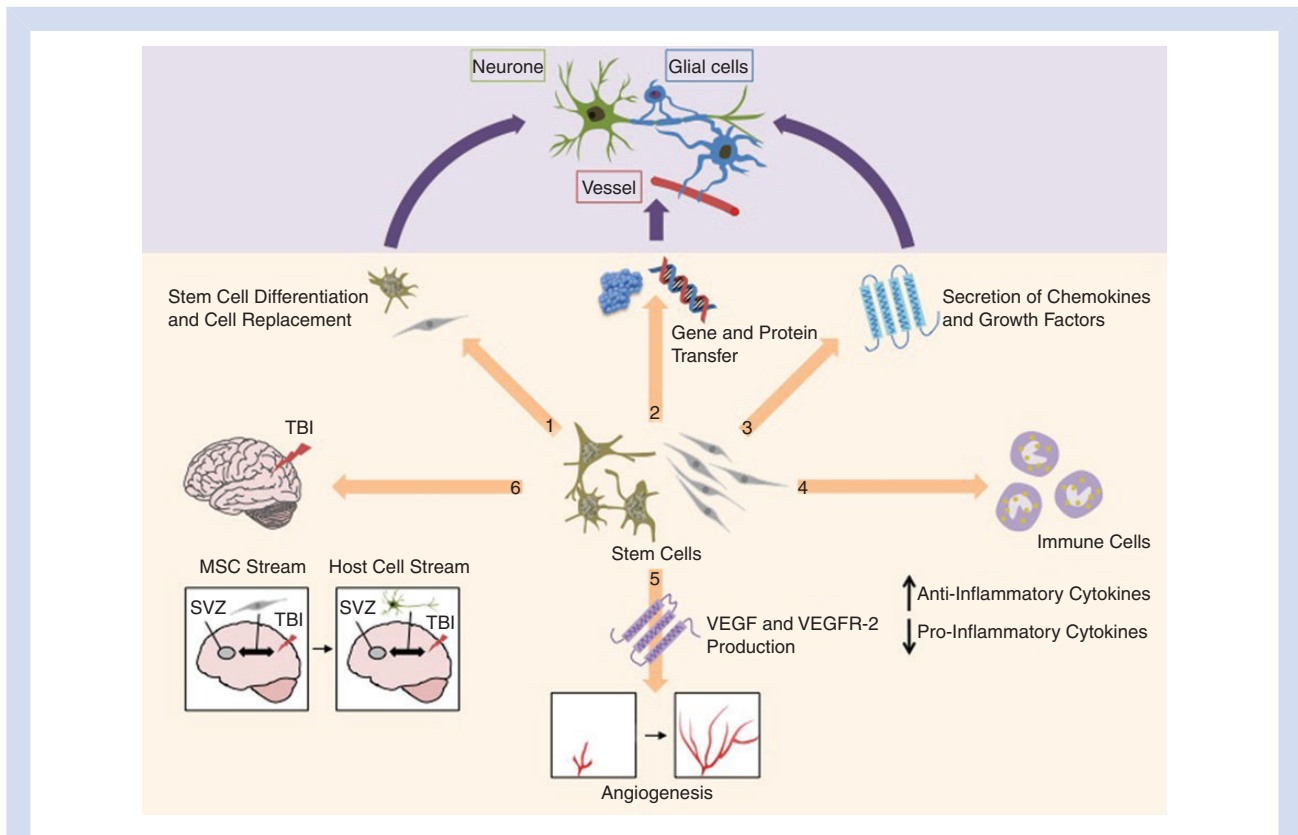


Fig 2 Potential Mechanisms of Action of Stem and Progenitor Cells in Traumatic Brain Injury. The therapeutic effect of stem and progenitor cells in traumatic brain injury (TBI) may be explained by the following mechanisms of action: 1) Differentiation of stem cells into loco-regional cell types and cell replacement although the long term engraftment rates are very low; 2) Stabilization of damage cells via gene and protein transfer by inter-cellular contact or fusion; 3) Increase of regional cell survival or proliferation via the secretion of chemokine and growth factors such as neurotrophic growth factor (NGF), brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF); 4) Reduction of oedema and inflammation caused by TBI, by enhancing, in the injured brain and the systemic circulation, the secretion of anti-inflammatory cytokines such as interleukin (IL)-10, and reducing the secretion of pro-inflammatory cytokines such as interferon- γ by immune cells; 5) Enhancement of angiogenesis and vasculogenesis in the ischaemic brain, by increasing VEGF secretion and the VEGF receptor (VEGFR)-2 expression; 6) And by development of pathways between the subventricular zone (SVZ) and the site of injury expressing high levels of extracellular matrix metalloproteinases and where transplanted stem cells implant initially. These transplanted stem cells act as pathways for the migration of host neurogenic cells. Once the 'biobridge' is formed, the grafted stem cells disappear and the host neurogenic cells persist, replacing the initial tasks of transplanted stem cells. BDNF, brain derived neurotrophic factor; FGF, fibroblast growth factor; IL, interleukin; NGF, neurotrophic growth factor; SVZ, subventricular zone; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2.

Delivery of stem cells

Two principal methods were used for MSC delivery: stereotactic injection^{14 17 21 23 24 27 28 35 37 38 40 44 48 49} and i.v. administration.^{19 20 25 29 32 33 39 41-43 45-47 49 51} Internal carotid artery^{29 34 50} and lateral ventricle^{30 31 36} injection methods were used infrequently. There is minimal literature comparing the delivery methods and outcomes. Mahmood and colleagues⁴⁵ found enhanced proliferation of transplanted cells in the ischaemic boundary zone and the SVZ when MSCs were administered stereotactically compared with i.v., but no difference was shown in terms of functional improvements. Lundberg and colleagues²⁹ found a higher brain engraftment of MSCs administered in the internal carotid artery vs those delivered i.v. but they did not evaluate the animal behaviour.

The timing of administration of MSC ranged from just before TBI²⁷ to one week after the injury,^{17 21 33 44} but most studies administered the cells 24 hours after TBI.^{19 20 25 29-32 34-37 39-42 45 46 49 50}

No study evaluated the effects of MSCs according to their timing of administration. Nevertheless, animal behaviour improvements have been shown even with late administrations.

Some studies used a scaffold, such as fibrin,^{22 28} matrigel,^{21 24} collagen,⁴⁴ or chitosan with gelatin,²³ to increase stem cell engraftment rates. The scaffold supplied extracellular matrix to maintain the viability of the implanted cells.⁵² In addition, it preserved the phenotype of the stem cell and had the capacity for spontaneous *in vivo* degradation. Guan and colleagues⁴⁴ showed that the use of collagen scaffold increased the number of MSCs in the lesion site, cell survival and neuronal outgrowth, while improving motor function and learning ability.

Main outcomes

The animals were followed for a variety of time-points, ranging from four hr⁴² to three months.^{17 33 37} Regardless of the route of administration, most studies showed improvements in motor function assessed by the modified Neurological Severity Score, the Rotarod test, Stepping or Balance Beam tests and learning ability assessed by the Morris Water Maze test.

Transplanted MSCs reduced cerebral lesion volume, in particular when delivered by stereotactic injection,^{17 21 23 24 27 28 30 40 51} either in the centre of the injury, the cortical area adjacent

to the injury, the ipsilateral hippocampus region, or the ipsilateral or contralateral ventricle. The assessment of cerebral lesion volume was done in almost all cases by histology; only a few studies used magnetic resonance imaging, positron emission tomography or a gamma camera to evaluate the effects of MSCs on cerebral lesion.^{42–44}

Transplanted MSCs delivered by stereotactic injection down-regulated the serum concentration of the pro-inflammatory cytokines, IL-1 β , IL-6, and TNF- α , 24 hours after their injection in the host brain.¹⁴ As release of pro-inflammatory cytokines after TBI can induce brain damage, this systemic effect of MSCs may contribute to improve neurological outcomes. MSCs also enhanced BDNF concentrations in the cerebrospinal fluid or in the injured brain, even more when previously transfected with the BDNF gene.^{30 33 37 45}

Transplanted MSCs delivered by stereotactic injection showed some ability to migrate into the ischaemic boundary zone,⁴⁵ the ipsilateral parenchyma,^{35–37 40} hippocampus,^{36 45} and SVZ,⁴⁵ and to the contralateral parenchyma to a lower extent.³⁶ Apart from the brain, i.v. administration of MSCs migrated to the heart, lung, liver, kidney, and spleen.^{19 20 25 32 33 45 46} Brain uptake in injured rats was very low, varying from 1.4%⁴¹ to less than 0.001%,⁴⁷ and even lower in uninjured animals,^{41 42} making it unlikely that cell engraftment would have any direct effect on outcomes. The survival rate of transplanted MSCs was also low: 14.4% at one week for Lu and colleagues,³⁴ 0.6% at one month and 0.16% at three months for Tajiri and colleagues.¹⁷ But, a few MSCs expressed neuronal markers, such as microtubule-associated protein-2 (between 4.1 and 8.4% at one week),³⁴ the neuronal nuclear antigen (between 2.9 and 5.6% at two weeks),^{25 34} and the neurone-specific class III beta-tubulin (Tuj-1).^{20 45} Others expressed the glial fibrillary acidic protein (between 7.1% and 15.8% at one week).^{25 34} In addition, proliferation of transplanted cells into the host brain was shown in several studies.^{17 38 49} Even more interesting, bone marrow derived MSCs delivered stereotactically and conditioned medium derived from these MSCs, increased NSC proliferation *in vivo* and *in vitro*, respectively.¹⁴ Rats exposed to the conditioned medium derived from MSCs exhibited a significant reduction in damaged brain volume assessed by histology, compared with rats exposed to control medium,⁵¹ and modifications of MSC conditioning (e.g. hypoxic exposure) had significant effects on the resulting damaged brain volume.⁵¹

These findings suggest that functional improvements after TBI may result from different mechanisms, other than cell replacement, such as local and systemic interactions between transplanted MSCs and cells involved in immunity or neural cell proliferation in the injured brain. Up-regulation of matrix metalloproteinase-9 in the injured brain¹⁷ and early restoration and preservation of cerebral blood flow⁴³ have also been suggested to be responsible for the therapeutic response.

Neural stem/Progenitor cells and NTERA2 cells

Cells origin, dose, and potency

NSCs and NPCs, the second most frequently used stem and progenitor cells for therapy in experimental TBI, were isolated from the postnatal mouse olfactory bulb and cerebellum,⁵³ embryonic murine ganglionic eminence,⁵⁴ embryonic rat hippocampus and forebrain tissue,^{40 55} adult rat hippocampus,⁵⁶ and from first-trimester embryonic human forebrain⁵⁷ (Supplementary Table). NTERA2 cells are human derived teratocarcinoma cells that differentiate into post-mitotic neurones when cultured *in vitro* with retinoic acid.⁵⁸

In small animal models of TBI, the number of cells used varied from 1.5×10^5 ⁵⁹ to 2.5×10^7 ⁶⁰ cells per kg body weight, with most doses between 10^5 and 10^6 . No study evaluated the effects of NSCs, NPCs or NTERA2 cells according to the dose administered, but individual studies with lower doses have not shown animal behaviour improvements when evaluated.⁵⁹

The potency of the stem cells was often enhanced by adding EGF and FGF-2 (also named basic FGF)^{57 60–68} or FGF-2 alone^{54 56 69–71} in the culture medium. In other studies, the stem and progenitor cells were transduced or transfected with a gene implicated in neuronal growth or differentiation.^{55 62 70 72 73} Philips and colleagues⁵⁵ found increased survival rate of pyramidal cells in the ipsilateral hippocampus but no clinical benefit, when delivering NGF transduced-NPCs compared with NPCs alone. Makri and colleagues⁷⁰ found an increased generation of neuronal cells compared with glial cells when delivering NSCs and NPCs transduced with the cell cycle exit and neuronal differentiation (Cend)-1 gene. Ma and colleagues⁶⁴ and Bakshi and colleagues⁷³ demonstrated improvements in motor function and learning ability in rats with higher survival rate and migration and neuronal differentiation of transplanted cells transfected with the BDNF gene or the glial cell-derived neurotrophic factor gene.

Small animal models

Traumatic brain injury was induced in adult Sprague-Dawley rats,^{56 57 59 61 63 65–68 72–74} C57BL/6 mice,^{53 54 60 69–71, 75 76} and Wistar rats.^{40 55 62 64} Cyclosporine A was usually administered in animals receiving human stem cells^{59 61 63 65 67 68 76} and in rats receiving mouse-derived stem cells.^{72 73} Wennersten and colleagues⁶⁷ showed in rats which received human NSCs and NPCs by stereotactic injection, that Cyclosporine A improved cell graft survival. The principal models of TBI used were the CCI,^{40 53 54 57 60–64 69 71 74–76} followed by FPI.^{55 59 66 68 72 73}

Delivery of stem cells

The delivery method used for NSCs, NPCs and NTERA2 cells was predominantly stereotactic injection. Skardelly and colleagues⁶³ studied the effects of pre-differentiated NPCs, adding an i.v. cell delivery to the stereotactic injection. They found no additional benefit of the i.v. injection. Wallenquist and colleagues⁶⁰ had higher transplanted cell survival when injected in the lateral ventricle, compared with stereotactic injection. No study compared i.v. to stereotactic injections alone.

The timing of delivery from TBI ranged from immediately after injury^{57 60 65 67 70} to one month later.⁵⁹ Cells were often delivered 24 hours^{40 55 63 66 68 73 75 76} or one week^{53 54 56 60 62 64 69 71 72 74} after TBI. Shear and colleagues⁷¹ found that stereotactic injection in the ipsilateral striatum, carried out two days or one week after TBI, led to better outcomes than two weeks after. Zhang and colleagues⁵⁹ also found no difference in motor and cognitive functions in rats receiving NTERA2 cells by stereotactic injection one month after the TBI.

The few pre-clinical studies using scaffolds such as fibronectin,⁶⁹ laminin,⁶⁹ or collagen⁵⁶ suggested improved outcomes, with long term transplanted cell survival and distribution into the injured brain.⁶⁹

Main outcomes

In the studies, the animals were followed for multiple time-points, from three days⁶⁸ to six months,⁶⁷ with most from one to three months.^{53 56 59–64 69–71 73 76} Interestingly, all studies showed improvements in motor function and learning ability. Transplanted cells were found to decrease the cerebral lesion volume⁶³ and shown to migrate to the ischaemic boundary

zone,^{55 60 61 63 65} ipsilateral parenchyma,^{40 53 54 56 59 61 62 65} hippocampus,^{54 55 64-67} SVZ,⁶¹ rostral migration stream,^{62 64} and olfactory bulb,⁶² and to the contralateral parenchyma to a lesser extent.^{61 65 72} Stem and progenitor cell engraftment persisted for up to six months.⁶⁷

The transplanted cell survival rate was low: 1.9% at two weeks for Harting and colleagues⁷⁴ and 4.1% at two months for Ma and colleagues.⁶² In addition, most studies failed to demonstrate the proliferation of transplanted cells in the host brain. Nevertheless, Zhang and colleagues⁵⁹ and Ma and colleagues⁶⁴ found that some transplanted cells expressed synaptophysin, a synaptic vesicle glycoprotein used for synapses quantification. Also, some transplanted cells expressed both neuronal markers, especially in the ipsilateral hippocampus,^{53 60 66 70} and glial markers.⁵⁶ A potential systemic effect of cells injected stereotactically or i.v. has not been demonstrated yet.

Similar to MSCs, these findings seemed to indicate improvements after TBI, despite limited stem cell engraftment. Increased angiogenesis in the injury border zone⁶³ and decreased amyloid precursor protein accumulation and α -smooth muscle actin expression in ipsilateral hippocampus (site of NSCs transplantation)⁶⁸ have been suggested to be responsible for some of the beneficial effects.

Embryonic stem cells

Embryonic stem cells were studied less frequently than NSCs, NPCs or MSCs, in the context of TBI (Supplementary Table). In all cases, they were isolated from the inner cell mass of murine blastocysts.^{77 78} The number of cells used varied from 3×10^5 ^{8 79 80} to 10^7 ⁷⁸ cells per kg body weight. Some embryonic stem cells were pre-differentiated before use,⁷⁹ and some were cultured with retinoic acid to further differentiate to neuron like cells,^{78 81} but without obvious biological or clinical improvements.

Studies were performed with Sprague-Dawley rats^{8 79 80} or C57BL/6 mice.^{78 81} Cyclosporine A was administered in two studies using murine embryonic stem cells in rats, without any benefit.^{8 80} Three models of TBI were used: the FPI,^{8 80} the cryogenic lesion^{78 81} and the CCI⁷⁹ model. All embryonic stem cells were delivered by stereotactic injection, between three days^{8 80} and one week^{78 79 81} after TBI.

Time-endpoints ranged between three⁷⁸ and seven^{8 80} weeks. Studies showed animal behavioural but no learning improvements. Transplanted embryonic stem cells migrated into the site of injury^{78 79} and to the ipsilateral hippocampus.⁷⁹ Some embryonic stem cells differentiated into neuron like cells,^{78 79 81} while others expressed glial markers.⁷⁹ Some implanted cells were phagocytosed by activated macrophages.⁸ Interestingly, Riess and colleagues⁸⁰ showed almost no detection of transplanted embryonic stem cells in the injured brain, but tumour formations were found seven weeks after their injection.

Multipotent adult progenitor cells

Multipotent adult progenitor cells are undifferentiated cells derived from the bone marrow (Supplementary Table). They are self-renewing and multipotent, precursors for adult progenitor cells, and able to differentiate to neural, endothelial and hematopoietic cells, *in vitro* and *in vivo*. Their surface markers include CD10, CD13, CD49b, CD49d, CDw90 and VEGFR-2.⁸²

Only one research team used human multipotent adult progenitor cells as a potential treatment of TBI in Sprague-Dawley rats^{83 84} and C57BL/6 mice.⁸⁵ The number of cells used in each experiment was around 10^6 , injected i.v. two and 24 hours after TBI.

The cells prevented increase in blood brain barrier permeability after TBI, primarily through modulation of immune cells,^{83 85 86} which was associated with increased anti-inflammatory macrophages (M2 phenotype) in the brain and higher IL-10 concentrations.⁸⁵

Endothelial progenitor cells

Endothelial progenitor cells, isolated from bone marrow and adipose tissue,^{87 88} were used in Sprague-Dawley⁸⁷ and Wistar⁸⁸ rats undergoing CCI (Supplementary Table). Chen and colleagues⁸⁸ found restored cerebral blood perfusion and increased cerebral microvasculature in the injured region at one week, when endothelial progenitor cells were administered six and twelve hours after TBI. Xue and colleagues⁸⁷ showed accumulation of endothelial progenitor cells in the injury site, their incorporation into capillaries, and reduced astrogliosis and inflammation, leading to better neurological outcomes.

Clinical studies

Only two clinical trials using stem and progenitor cells as treatment for acute or sub-acute TBI have been published.^{89 90} Cox and colleagues⁸⁹ conducted a prospective, non-random, open label, phase 1/2 clinical trial (NCT00254722) on 10 children aged five to fourteen yr with a post-resuscitation GCS of five to eight. They administered i.v. 6×10^6 autologous bone marrow derived mononuclear cells (from which MSCs and multipotent adult progenitor cells are derived) per kg body weight within 48 hours after brain injury and conducted a six months follow-up. They found no episodes of post-injury seizures, refractory intra-cranial pressure, alteration in cerebral perfusion pressure, or new ischaemic event. Every patient showed some neurological improvement, but only three patients recovered completely. No significant brain morphologic change was found by magnetic resonance imaging between one and six months. Tian and colleagues⁹⁰ conducted a prospective, non-random, open label, phase 1/2 study, on 97 patients in the sub-acute phase of TBI. They administered a mean dose of 4×10^6 autologous bone marrow derived mononuclear cells, intrathecally by lumbar puncture, within two months after the injury and conducted a 40 day follow-up. They found no serious complications or adverse events. Twenty seven patients showed improvements in motor functions, and 11 of 24 patients in vegetative state showed improvements in consciousness. The outcome was better for younger patients and for patients who received therapy earlier after injury.

Three clinical trials are currently ongoing. NCT01851083 is a randomized controlled phase 1/2 trial, studying the effects of autologous bone marrow derived mononuclear cells, delivered i.v. in children aged five to seventeen years, with a hospital admission GCS of three to eight. The primary outcome is improvement by brain magnetic resonance imaging, and the secondary outcome is improvement in functional and neurocognitive deficits. One potential implication of this study will be the ability to determine the relationship between neurological status and brain morphology. NCT01575470 is an open label phase 1/2 trial studying the effects of autologous bone marrow derived mononuclear cells delivered i.v. in adults aged 18-55 yr with a post-resuscitation GCS of five to eight. The primary outcomes are the number of neurological events and cerebral vascular accidents, and the secondary outcomes are the global functional status and the level of the disability. And lastly, NCT02028104 is an open label phase 1/2 trial, studying the effects of autologous bone marrow derived mononuclear cells, delivered intrathecally in brain

injured patients aged six months to 65 yr. Primary and secondary outcomes are the change in clinical symptoms and the level of the disability rating scale. One potential implication of this study will be the ability to determine the optimal route of deliver of the stem and progenitor cells.

Clinical trial and research perspectives

These early clinical trials are encouraging, in particular because they have showed safety with the absence of serious side-effects. There is strong rationale for the use of MSCs, NSCs or NPCs as treatment for TBI. Currently, the potential use of NSCs and NPCs in clinical trials is mainly limited by the difficulty generating large quantities of NSCs or NPCs for administration. Whereas, the potential use of MSCs is very promising based on their relative ease of procurement, immune-privileged property allowing an allogeneic source, and well documented safety profile.⁹¹ Surprisingly, all the current clinical trials have used bone marrow derived mononuclear cells as treatment after TBI, primarily because of the autologous source of the cells. One wonders whether similar or improved neurological benefits can be achieved with MSCs compared with a similar dose of mononuclear cells.

However, questions still remain concerning the optimal route and timing of delivery of the cells after TBI and the monitoring parameters utilized for safety and efficacy. Stereotactic injection, although feasible, is highly invasive and will require the skills of a neurosurgeon, whereas i.v. and intrathecal injections are much more accessible delivery routes. As the long term engraftment rates are very low, i.v. delivery may be as efficacious as stereotactic or intrathecal delivery. Most pre-clinical trials have delivered the cells early after TBI to suppress the initial inflammatory response and activation of the cells of innate and adaptive immunity. There is minimal pre-clinical evidence of benefit when stem and progenitor cells are delivered more than one week after TBI.⁹² Initial monitoring for safety and efficacy should combine magnetic resonance imaging for its ability to show cerebral lesion and perfusion, biological parameters such as the systemic concentrations of pro- and anti-inflammatory cytokines, and multiple neurological tests allowing a comprehensive overview of the patient's neurological state. Although functional recovery of the hippocampus may be an attractive endpoint for clinical trials, many of the effects of stem and progenitor cells are nonspecific, such as the stabilization of the blood-brain barrier preventing excessive cerebral oedema.

Significant obstacles still remain for conducting randomized controlled trials for efficacy. The optimal cell type, dose, delivery route, and timing of administration and which monitoring parameters would be necessary and for how long needs to be determined in humans. In addition, the cost of conducting such clinical trials, specifically the cost of generating and storage/processing of the stem and progenitor cells before administration, is significant. Lastly, although using adult stem cells and an autologous source will diminish the long term risk of iatrogenic tumour formation, patients in clinical trials will generally receive 5 to 10×10^6 cells kg^{-1} or up to 1 billion cells per treatment. There is no adequate monitoring device (i.e. CT or MRI scan) yet, which can differentiate iatrogenic tumour foci from inflamed or injured tissue immediately.

Conclusion

TBI is a major public health issue in need of innovative treatment options. Cell-based therapy may be a promising approach. Pre-clinical research in small animal models of TBI has paved the

way for early phase 1/2 clinical trials. Nevertheless, more studies are needed to address the optimal stem cell used, dose and delivery method. And above all, more preclinical work is necessary to further understand the mechanisms underlying the therapeutic effect of stem and progenitor cells in the injured brain.

Supplementary material

Supplementary Material is available at *British Journal of Anaesthesia* online.

Authors' contributions

Study design/planning: S.G., J.W.L.

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Revising paper: All authors.

Declaration of interest

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