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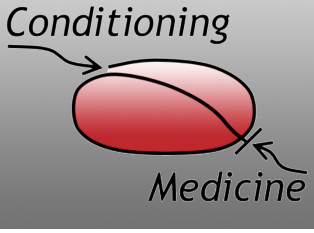

REVIEW ARTICLE | OPEN ACCESS

Cell therapy and conditioning medicine for stroke

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Transplanted stem cells exhibit modest engraftment in the stroke brain. Despite this restricted graft survival, transplanted stroke animals display functional recovery owing in part to stem cells' secretion of therapeutic molecules. Clinical trials of cell therapy in stroke patients commenced in 1998 generating a solid safety profile but efficacy remains elusive. Indeed, two recent stroke clinical trials while demonstrating safety, failed to produce significant clinical functional recovery. Here, we discuss the potential of conditioning stem cells to prepare them for the harsh microenvironment of the ischemic brain. In particular, we present hypoxic conditioning or oxygen deprivation as a conditioning strategy for generating a hi-breed of stem cells with enhanced survival and function in the stroke brain. We advance here the concept of combining cell transplantation and conditioning medicine towards developing an improved stem cell-based stroke therapeutic.

Keywords: Stroke, Stem cell therapy, Regenerative medicine, Hypoxia, Microenvironment

Cell transplantation and stroke

Pioneering investigations of stroke cell therapy initiated in the late 1980s show the survival of mouse fetal neocortical grafts in the ischemic cortex of aged rats (Mampalam et al., 1988; Sharp, 1993). These grafted fetal cells integrated with ischemic tissues by receiving afferent fibers as well as the vascularization of the healthy tissue of the host, thus aiding the brain in responding to sensory stimulation and increasing its metabolic activity Grabowski et al., 1992a; 1992b; 1993). Equally a milestone discovery, stroke animals transplanted with fetal striatal cells displayed improvements in cognitive tasks (Nishino et al., 1993; Aihara et al., 1994).

Over the last forty years, preclinical and clinical evidence shows survival, migration, differentiation, and functional integration of transplanted cells in the ischemic brain. Such graft persistence coincides with brain circuitry remodeling, anatomical reconstruction, and neurochemical, physiological, and behavioral recovery has been demonstrated (Sharp, 1993; Borlongan et al., 1997). Therapeutic effects of cell transplants in stoke may entail transplanted cells replacing ischemic or dead cells. Compelling evidence also demonstrates that therapeutic substances secreted by the grafted cells may promote angiogenesis, vasculogenesis, neurogenesis, and anti-inflammation, among other regenerative processes (Bliss et al., 2007; Liu et al., 2014; Zhang et al., 2016; Stonesifer et al., 2017). Altogether, these brain repair processes served as the basis for the introduction of cell transplantation therapy for stroke in the clinic. Allogeneic transplantation of fetal cells paved the way for the initial clinical trials of cell therapy in Parkinson's disease but these cells encountered logistical and ethical challenges. Circumventing these lab-to-clinical hurdles led to the development of human teratocarcinoma cells modified to differentiate into postmitotic neurons, eventually transplanted into stroke patients (Borlongan et al., 1998; Kondziolka et al., 2000). Subsequently, along the lines of finding a non-fetal and non-cancerous transplantable cells, embryonic stem cells, induced pluripotent stem cells, and cells derived from adult

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tissues, including those from bone marrow, umbilical cord and adipose tissue, represent viable donor cells for transplantation therapy in stroke (Sun and Kurtzberg, 2015; Napoli and Borlongan, 2017; Bateman et al., 2018).

Focusing on adult tissue-derived stem cells

Bone marrow has become the preferred adult tissue stem cell source due to its solid safety profile (Tang et al., 2007; Borlongan 2016a; 2016b; Napoli and Borlongan, 2016; 2017; Napoli et al., 2018). Mesenchymal stem or stromal cells (MSCs), mononuclear cells (MNCs), endothelial progenitor cells (EPCs), SB623, multipotent adult progenitor cells (MAPCs), and multilineage-differentiating stress-enduring cells (MUSE), are some characterized cell populations derived from the bone marrow and engineered stem/progenitor cells (Yasuhara et al., 2008; 2009; Gnecchi and Melo, 2009; Li et al., 2015; Uchida et al., 2015). These bone marrow-derived stem cells display multipotent cell capacities in preclinical models of stroke (Joyce et al., 2010; Borlongan 2011; Borlongan et al., 2011; Kocsis and Honmou, 2012; Eckert et al., 2013; Rowart et al., 2015). Furthermore, after transplantation, grafted stem cells attenuate histological and behavioral deficits in vivo (van Velthoven et al., 2014). Preclinical data demonstrate bone marrow-derived stem cells' potential as a powerful therapeutic, paving the way for clinical trials of cell therapy. A study intravenously administered autologous bone marrow MSCs to patients 4-weeks post-stroke resulting in improved neurologic outcomes that were initially observed alongside no adverse effects, but these benefits diminished by 12-months post-transplant (Bang et al., 2005). An open-labeled trial administered autologous bone marrow MNCs intravenously 24-72 hours after stroke onset also showed safety as well as improved functional recovery that lasted six months after transplantation (Savitz et al., 2011). A phase II, multicenter, parallel group, randomized, and blinded trial intravenously transplanted autologous bone marrow MNCs at an average of 18.5 days post-stroke. Compared to the previous study, data indicated that this treatment was safe, however no functional improvements were observed (Prasad et al., 2014). Furthermore, an open-labeled trial administered a subset of cluster of differentiation (CD)34^+ bone marrow MNCs intra arterially in a 7-day window post stroke. This also demonstrated safe application as well as increased functional improvements throughout a six-month period (Banerjee et al., 2014). More recent clinical trials have reported consistent safety among cell transplantation, however mixed outcomes in terms of functional recovery. Data indicate a lack of efficacy in intravenous transplantation of MAPCs in acute stroke patients (Hess et al., 2017) and in chronic stroke patients after intracerebral administration of SB623 (Steinberg et al., 2016a; 2019b; SanBio).

Based on the previously discussed interim clinical trials, transplantation of bone marrow stem cell derivatives, especially MSCs and MNCs, in stroke is safe. However, the efficacy of the treatment remains elusive. Conclusive interpretations drawn from clinical data are hindered by a small number of patients enrolled in the trials in addition to the open-labeled approach. Vis-à-vis comparisons between hindered trials are difficult due to the difference in donor cells and varied clinical transplant protocol, such as cell dose, timing, and delivery routes. MSC phenotypic markers include SH-2 and SH-4 (Bang et al., 2005) specific flow cytometric antibodies comprise of CD3, CD14, CD16, CD19, CD20, CD34, CD45, CD56, Lin 1, and CD133-2 (Savitz et al., 2011) although some studies are limited to CD34 and CD45 (Prasad et al., 2014) or focus on $CD34^+$ cells in magnetic cell isolation procedures (Banerjee et al., 2014). MAPCs are defined as c-Kit^+, $CD0^+$, $CD13^+$, $CD31^+$, $CD44^-$, MHC-I^-, $Thy1^-$ (Sohni and Verfaillie 2011; Hess et al., 2017), while SB623 are notched-induced MSCs (Steinberg et al., 2016; 2019; Dezawa et al., 2004; Tajiri et al., 2013; 2014). In addition to various administration routes across the trials (Bang et al., 2005; Savitz et al., 2011; Banerjee et al., 2014; Prasad et al., 2014; Steinberg et al., 2016; 2019; Hess et al., 2017), therapeutic windows span from acute to chronic stroke stages.

Safe, but not effective in clinical trials

A primary reason for the lack of success of clinical trials to achieve efficacy standards is the inconsistency in the laboratory and clinical stem cell transplant protocols. The efficacy seen in the laboratory, which follows specific dosing and timing of transplants, is not strictly adhered in the clinic. In preclinical studies, an effective dose administered intravenously is approximately 4 million cells in stroke rats weighing 250 g and 840 million cells in a stroke patient weighing 75 kg (Diamandis and Borlongan, 2015). However, most clinical studies use cell counts far below the demonstrated effective dose (Bang et al., 2005; Banerjee et al., 2014; Prasad et al., 2014). Notably, clinical trials that gave stroke patients the preclinical cell dose showed improvements. In intracerebral transplants, the effective dose in stroke rats is 200,000 cells, which equates to about 56 million cells in stroke patients, yet clinical doses (2.5 million and 5 million) administered were far below the recommended dose, potentially explaining the lack of efficacy (Steinberg et al., 2016; 2019). The efficacious cell dose (400 million to 1200 million cells) indicated in stroke rats was maintained in the MAPC trial (Hess et al., 2017), but efficacy was not attained (Kenmuir and Wechsler, 2017). Posthoc analysis uncovered that patients who received the MAPC transplant within 36 hours displayed functional improvements (Athersys 2015), which resembled the preclinical study (Mays et al., 2010) and are now being pursued in another MAPC trial (Doeppner and Hermann, 2010). Clinical trials should strictly follow previous preclinical guidelines for optimal dosing, timing, and route to achieve maximum efficacy.

Strategies to enhance stem cell survival and function in stroke

The clinical trial design of stem cell therapy has highlighted the logistics of the transplant treatment, yet it may have disregarded basic discoveries about the properties of stem cells. The standard thought process when visualizing a stem cell clinical product always involves a clear set of respective phenotypic markers plus a firm grasp of the functioning of the stem cells. Acquiring clinical grade stem cells should prevent conventional product release rules of either homogeneous populations of cells or a reliable generation of the same stem cell population. Additionally, the clinical transplant regimen should expand upon the postulated methods from the laboratory, involving growth factor secretion, cell replacement, and promotion of endogenous brain repair processes (Duffy et al., 2009; Ning et al., 2011; Ishikawa et al., 2013; Anderson et al., 2016; US National Library of Medicine, 2019), as these methods can simultaneously function to contest cell death pathways seen in stroke (Sozmen et al., 2012; Puyal et al., 2013; Schweizer et al., 2013). Overall, stem cell therapy can enhance its therapeutic potential when it does not act independently but rather combined with tissue plasminogen activator (tPA), other neuroprotective drugs, biomaterials (Incontri et al., 2018), or thrombectomy (see below), along with standard stroke procedures for rehabilitation (Polgar et al., 1997; Borlongan et al., 2015).

Additionally, it is important to analyze both the safety and efficacy of the stem cells in preclinical stroke models. This includes the analysis of their tissue formation capacity, persistence, and cell fate after transplantation. When a stem cell differentiates, it may induce a damaging immunomodulatory or inflammatory response that is toxic or cause tumorigenesis.

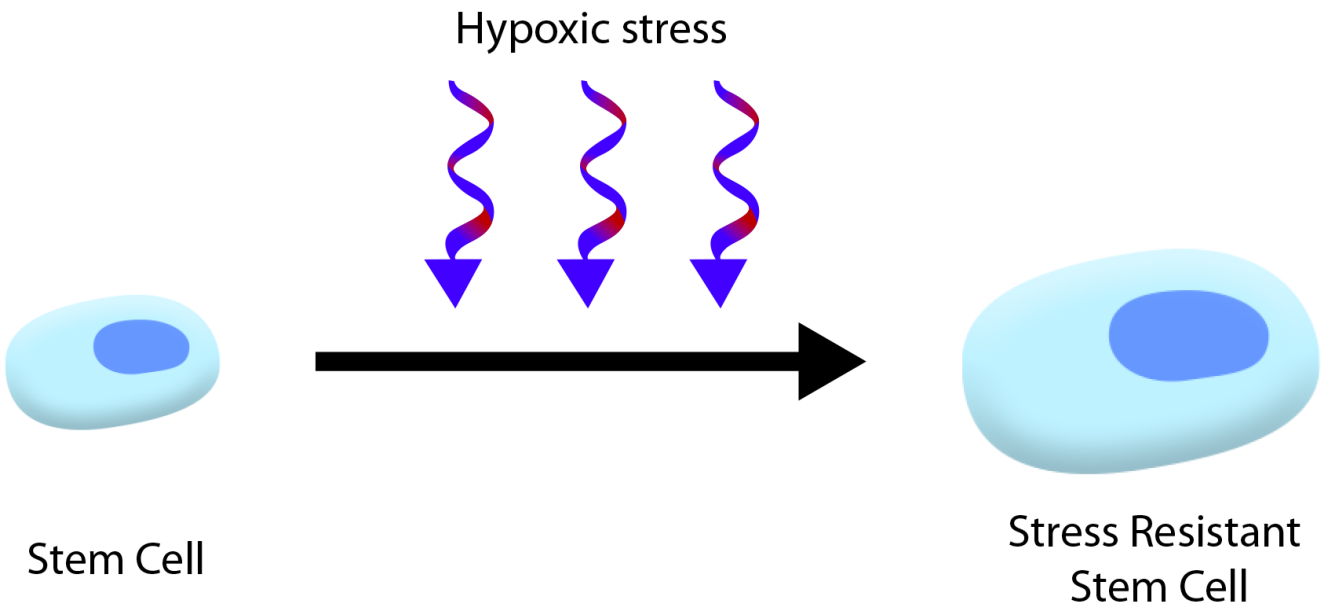

Figure 1. **Conditioning of stem cells.** Exposing cultured stem cells to hypoxic stress and oxygen deprivation prior to transplantation enhances ischemic tolerance. These conditioned stem cells may be more tolerant to the harsh environment of the ischemic brain, allowing them to survive and function better than non-conditioned stem cells.

Thus, extra precautions should be taken when using genetically engineered stem cells such as SB623 and immortalized cells such as CTX03 (Borlongan, 2016; Napoli and Borlongan 2017). Additional safety precautions are also warranted when using cryopreserved cells or extracellular matrix-loaded cells. The use of delivery devices including nanoparticles, exosomes, extracellular vesicles, microRNAs, mitochondria, and other cellular components also requires further safety deliberations (Chen et al., 2017; Lamanna et al., 2017; Labriola et al., 2018; Liebeskind et al., 2018; Nguyen et al., 2018; Venkat et al., 2018). Further advancements have paved the way for the use of cell-derivative and cell-free compositions over cell-directed transplantation for stroke treatment that requires successful safety outcome evaluations.

Conditioning medicine as a novel approach to improve cell therapy outcomes

Conditioning medicine is defined as a strategy designed to increase the tolerance level of an organ against injury (Yang et al., 2016). In the case of stroke, brain exposure to a conditioning paradigm designed to enhance ischemic tolerance results in brain protection against a subsequent stroke. There are several forms of conditioning, including physical (exercise), pharmacologic (drugs), biologics (stem cells), and a sublethal form of the injury itself. The timing of conditioning can be initiated prior to (pre), during (per), or after (post) the injury. The target site of conditioning can be direct to the organ or remotely. Exposure of cultured stem cells to a hypoxic environment or oxygen deprivation prior to transplantation represents a robust strategy in conditioning the cells to the non-conductive tissue environment of the stroke brain (Figure 1). Hypoxic preconditioning increases cell viability and paracrine activity, specifically vascular endothelial growth factor-A, stromal cell-derived factor-A, interleukins, and tumor necrosis factor (Li et al., 2010; Andreeva et al., 2015; Bader et al., 2015; Veighey, 2020; Zhao et al., 2020). A similar stroke-like stressful event upregulates trophic factor secretion in stem cells (Hou et al., 2017; Bachmann et al., 2020). Moreover, hypoxia preconditioning increases the survival of transplanted MSCs while upregulating the therapeutic paracrine effects of injured cells (Huang et al., 2020). In parallel, hypoxic preconditioning of MSC-derived conditioned medium drives microglial cells to polarize into an anti-inflammatory phenotype thereby protecting against stroke (Zhang et al., 2019; Yu et al., 2021). The participation of exosome in such preconditioning has been suggested (Deng et al, 2018). Altogether, these results suggest that hypoxia preconditioning improves stem cell therapy in ischemic stroke.

Conclusion

Stem cell transplantation for neurological disorders is safe, but its efficacy remains in question. Several types of stem cells have been explored in the laboratory, and a few of these cells have advanced to clinical trials, with varying modest results. Strategies designed to improve the clinical outcomes of stem cell transplantation have entailed strict adherence to stem cell protocols outlined in preclinical studies to achieve consistent results. Adjunctive application with other available treatments, such as neuroprotective drugs, biomaterials, tPA, or mechanical thrombectomy, may enhance the therapeutic effects of stem cells. With this in mind, exposure of stem cells to a hypoxic environment or oxygen deprivation may improve transplanted stem cell survival and function in the stroke brain. Combination of cell transplantation and conditioning medicine represent a new field that will likely benefit the field of regenerative medicine for stroke and other related neurological disorders.

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