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以幹細胞及G-CSF治療腦急性損傷的神經發炎，可以合併治療之後的老化現象

Stem cells and G-CSF for treating neuroinflammation in traumatic brain injury: aging as a comorbidity factor

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Abstract

Traumatic brain injury (TBI), often called the signature wound of Iraq and Afghanistan wars, is characterized by a progressive histopathology and long-lasting behavioral deficits. Treatment options for TBI are limited and patients are usually relegated to rehabilitation therapy and a handful of experimental treatments. Stem cell-based therapies offer alternative treatment regimens for TBI, and have been intended to target the delayed therapeutic window post-TBI, in order to promote “neuroregeneration,” in lieu of “neuroprotection” which can be accomplished during acute TBI phase. However, these interventions may require adjunctive pharmacological treatments especially when aging is considered as a comorbidity factor for post-TBI health outcomes. Here, we put forward the concept that a combination therapy of human umbilical cord blood cell (hUCB) and granulocyte-colony stimulating factor (G-CSF) attenuates neuroinflammation in TBI, in view of the safety and efficacy profiles of hUCB and G-CSF, their respective mechanisms of action, and efficacy of hUCB+G-CSF combination therapy in TBI animal models. Further investigations on the neuroinflammatory pathway as a key pathological hallmark in acute and chronic TBI and also as a major therapeutic target of hUCB+G-CSF are warranted in order to optimize the translation of this combination therapy in the clinic.

Keywords

Brain injuries; Stem cells; Aging

The wars in Iraq and Afghanistan have highlighted traumatic brain injury (TBI) as a significant unmet clinical need, characterized by high mortality and severe morbidity.[1–4] Although traditionally considered as an acute event, TBI has now been recognized as being accompanied by chronic pathological symptoms, notably secondary cell death mediated by long-lasting neuroinflammation, and closely associated with life-long behavioral deficits.[5, 6] To date, treatment for TBI is limited,[7] with patients largely relegated to rehabilitation therapy.[8–12]

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In view of the rampant secondary cell death mediating the progression of TBI, novel treatments have targeted the delayed therapeutic time window post-TBI termed as "neuroregeneration" as opposed to the narrow "neuroprotection" window relegated to the acute TBI phase.[4, 13] A major component of regenerative medicine is stem cell-based therapeutics which have been shown effective in animal models of neurological disorders, including TBI[4, 14–17] and have reached limited trials in the clinic.[18] Fetal stem cells, cancer-derived neuron-like cells, embryonic stem cells, induced pluripotent stem cells, and adult stem cells, such as umbilical cord blood, bone marrow stromal cells, amnion cells, have been examined for their safety, efficacy, and mechanisms of action for treating brain diseases.[4, 14–18, 19–24] Our group, and several others, have assessed the clinical utility of human umbilical cord blood (hUCB)-derived cells in stroke, Parkinson's disease, Huntington's disease, cerebral palsy, and TBI.[20, 25–28] Limited clinical trials of hUCB cells are being explored in cerebral palsy, inborn metabolic disorders, and stroke.[26, 29–31]

In an effort to initiate clinical trials of hUCB cell therapy for TBI, translational research is necessary to determine the optimal transplant regimen and to provide a better understanding of the mode of action of stem cells in regenerating the injured brain. To this end, demonstrating a well-defined stem cell source is a basic translational gating item for quality assurance and quality control of graft origin, and also for validity and reproducibility of experimental outcomes. In the case of hUCB cells, there remains a paucity of research which identifies the appropriate cell population that is safe and effective for transplantation. There are reports implicating that the mononuclear fraction of hUCB exerts neuroprotective effects via multi-pronged neuroregenerative pathways including anti-inflammation and enhanced neurogenesis[32–34], while MSCs derived from hUCB have also been shown to promote functional benefits by increasing angiogenesis and vasculogenesis.[35–37] In addition to identifying the optimal transplantable hUCB cell phenotype, low graft survival has been documented in the TBI brain, which may be due to the not-so-conducive host tissue likely created by the secondary neuroinflammatory response.[38–40] While robust graft survival may not be necessary to induce behavioral recovery, the alternative mechanism of graft-induced by-stander effects still requires for the hostile microenvironment to be abrogated if improved clinical outcome is desired. Accordingly, the concept that stem cell therapy can be enhanced by rendering a receptive microenvironment (*i.e.*, less neuroinflammatory) appeals to advancing regenerative medicine for treating the injured brain.

Repurposing of old drugs poses as a logical approach when contemplating on testing adjunctive therapies for stem cell transplantation. An appealing drug candidate is the granulocyte-colony stimulating factor (G-CSF), an essential member of the hematopoietic growth factor family, which has received much attention over the last decade for its neuroprotective effects in animal models of stroke[41] and Alzheimer's disease[42] via its action on reducing peripheral inflammation while stimulating neurogenesis.[43, 44] Currently, a limited clinical trial is underway for testing G-CSF in TBI patients (personal communication with Dr. Juan Sanchez-Ramos, USF/VA). Notwithstanding inconsistent efficacy results with some studies showing significant functional recovery, while others reporting small incremental behavioral benefits G-CSF in TBI animals [e.g. 45,46], the translation of G-CSF

for TBI in the clinic is based on the drug's safety profiles for its indication in acute ischemic stroke and Alzheimer's disease.[41, 42, 47]

Combining stem cell therapy and G-CSF for treating neuroinflammation in traumatic brain injury

The potential of combining stem cell transplantation with G-CSF to achieve improved therapeutic outcome in TBI has been recently examined.[48] Using a controlled cortical impact (CCI) model of moderate TBI in adult rats, we demonstrated that while monotherapy with hUCB or G-CSF promoted behavioral recovery, combined therapy of hUCB+G-CSF enhanced functional improvement compared to hUCB transplantation or G-CSF administration alone. In line with the concept of reducing neuroinflammation within the injured brain, co-administration with G-CSF might have produced a conducive microenvironment for the transplanted hUCB cells to integrate with the host tissue.[49] Immunohistochemical staining with OX-6, which labels MHCII+ activated microglia, revealed that hUCB+G-CSF reduced TBI-induced neuroinflammation in gray and white matter areas.[48] In addition, G-CSF might have directed the migration of endogenous stem cells mobilized from the bone marrow to the site of injury[41–44, 46–49] and/or the grafted hUCB cells might have released growth factors secreted by hUCB grafts, and such combined regenerative pathways elicited a much more beneficial functional outcome than a single, stand-alone treatment.[48]

Additive and synergistic effects likely mediated the improved outcomes produced by combined hUCB+G-CSF in TBI animals. Indeed, hUCB alone treatment reduces inflammation and promotes neurogenesis and angiogenesis,[50, 51] while G-CSF alone enhances neurogenesis.[42] Interestingly, the reduction in neuroinflammation exerted by hUCB+G-CSF therapy coincided with elevated neurogenesis in the dentate gyrus and subventricular zone of the hippocampus while increasing the survival of hippocampal neurons in TBI rats.[48] These findings suggest that hUCB+G-CSF synergistically diminished TBI-induced neuroinflammation and stimulated endogenous neurogenesis, while reducing cell death in the injured brain and promoting functional recovery in TBI animals.[48]

As with any drug therapy, the blood-brain barrier penetrance and the ligand-receptor mechanism become translational gating items when advancing GCSF therapy to the clinic. Of note, G-CSF is able to cross the blood-brain barrier and reach neurons and glial cells through the G-CSF receptor,[52] and confers inhibitory actions upon pro-inflammatory cytokines while upregulating neurogenesis.[41, 44, 52–55] In combination with stem cell therapy, G-CSF maintains stemness and directs differentiation of hUCB cells.[56] In parallel, G-CSF directed migration of mobilized bone marrow cells may interact with the transplanted hUCB cells involving a paracrine secretion of trophic factors, growth factors, chemokines and immune-modulating cytokines (also termed "bystander effects" of transplanted stem cells as noted above).[57, 58] Such combination therapy of hUCB+G-CSF may thus entail a multipronged regenerative mechanism involving a receptor-mediated transport machinery with paracrine action in providing improved therapeutic outcome for TBI.

Aging exacerbates TBI-induced neuroinflammation: potential for cell therapy and G-CSF

Although the current theme of TBI has focused on our returning soldiers from the wars in the Iraq and Afghanistan, the aging population represents an equally large number of patients who suffer from TBI.[59, 60] Thus, studies designed to incorporate aging as a comorbidity factor in TBI may provide a better understanding of the disease pathology, as well as the treatment regimen for this key patient population. In a recent study, mild TBI was performed in young (6-month-old) and aged (20-month-old) rats, and then transplanted intravenously (3 hours later) with 4×10 (6) human adipose-derived stem cells (Tx), conditioned media (CM), or vehicle (unconditioned media).[4] The results showed significant recovery of motor and cognitive functions in young, but not aged, Tx and CM groups. Imaging analyses of hADSCs deposition revealed robust migration of Tx in the young spleen, but poorly reached the aged spleen. Moreover, while significant decrements in cortical damage and hippocampal cell loss were seen in both Tx and CM groups in young rats, only partial neuroprotection was observed in the aged rats and mainly in the Tx group but not the CM group. These findings suggest that while cell therapy appears effective for TBI, the reduced efficacy in aged rats, likely due to defective migration of the cells to the spleen, warrants optimization studies to improve the outcome of this treatment in the aging population. These suboptimal therapeutic effects solicit a similar combination therapy to supplement the functional benefits of stem cells transplantation. Because the spleen is a major source of systemic inflammation associated with neurological disorders, such as stroke and TBI,[61–63] and is known to participate in the neuroprotective effects of cell therapy in TBI,[64, 65] a drug that targets spleen-mediated inflammation is a logical candidate for such combination therapy with stem cells. As noted above, G-CSF stands as a robust neuroprotective agent, and additionally with highly potent anti-inflammatory effects.[66–69] Recognizing that the aging brain when exposed to TBI may present with widespread inflammation, treatment with G-CSF may not only reduce the systemic inflammation, but may also harness a conducive host-brain microenvironment allowing the transplanted stem cells to exert their maximal therapeutic benefits.

Future directions

A progressive histopathology and long-lasting behavioral deficits characterize TBI. Rehabilitation therapy, and a handful of experimental treatments, remains the often utilized option for treating TBI patients.[70] The advent stem cell-based therapies offers alternative treatment regimens for TBI, but may require adjunctive pharmacological treatments, especially with the recognition that aging exacerbates TBI pathology rendering the brain hostile to stem cell survival and to the functional effects of transplanted stem cells. The combination therapy of hUCB+G-CSF appears beneficial in TBI animal models. The safety and efficacy profiles of hUCB and G-CSF, and their respective mechanisms of action, point to their entry into the clinic. Further investigations on the neuroinflammatory pathway as being a key TBI acute and chronic pathological hallmark, but also as a major therapeutic target of hUCB+G-CSF should optimize the application of this combination therapy for TBI.

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