Immune modulation as a tool in promoting recovery from traumatic brain injury and stroke

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Abstract

Traumatic brain injury and ischemic stroke are two heterogeneous pathologies. However, at the cellular and tissue levels, their etiologies bear striking similarities. Both result from an initial insult that is rapidly exacerbated by a neuroinflammatory as well as a subsequent systemic inflammatory response. Moreover, both traumatic brain injury and stroke appear to up-regulate neurogenesis as well as draw neural progenitors to the damaged cerebral parenchyma via chemotaxis. This paper explores the concept of immune system modulation as a means of regulating the inflammatory pathways involved in the immediate aftermath and weeks following an injury event. Recent research supports the idea that inhibition of the systemic inflammatory response in the days following injury, as well as potentiation of inflammation in subsequent weeks may promote neuronal progenitor differentiation and migration and could improve functional outcome among patients suffering from stroke or traumatic brain injury.

Introduction

Ischemic stroke is currently a leading cause of worldwide mortality as well as the leading cause of permanent disability among adults.[1,2] Likewise, traumatic brain injury is another leading cause of mortality as well as a major cause of functional disability in the United States.[3] The proximal causes of ischemic stroke and traumatic brain injury are clearly different. However, the underlying pathology is remarkably similar at the molecular, cellular, and tissue levels. Both conditions result in an initially destructive inflammatory pathology. However, both stroke and traumatic brain injury result in upregulation of endogenous neurogenesis as well as chemotaxis-driven neuronal progenitor migration to the injured site. While thrombolytic therapy with tissue plasminogen activator (tPA) is the current standard of care for qualifying stroke patients, very few patients are eligible to receive it, and there is currently no clinically accepted treatment for traumatic brain injury other than the management of symptoms and supportive care.[4]

The neuroinflammatory cascade associated with these two pathologies appears initially destructive and promotes a systemic inflammatory response in the acute phase of injury.[5] However, it also appears subsequently beneficial in the chronic phase of injury by facilitating neural progenitor migration as well as localization to the area of injury.[6] Studies have shown that blocking neuroinflammation in the acute phase of injury greatly reduces functional impairment as well as neural damage, and that blocking this same pathway in the chronic period tends to detract from functional recovery.[7] Therefore, it can be posited that the management of both stroke and traumatic brain injury would potentially benefit from the inhibition of inflammation in the hours and days following injury as well as from promotion of the localized inflammatory process in subsequent weeks.

Injury to the cerebral parenchyma begins within minutes whether the initial insult is traumatic or ischemic in nature.[8] Cellular death may continue for hours, days, and weeks following the primary injury.[9] Cytokines released from the damaged parenchyma as well as damaged vasculature initially promote a local neuroinflammatory response, followed by a systemic inflammatory response in the hours and days following vascular occlusion or traumatic brain injury, and amplifies damage caused by the initial lesion.[10,11] Despite the initially detrimental immune response to injury, proinflammatory factors released from damaged tissue as well as by the general inflammatory response ultimately promote neurogenesis and enhance neural progenitor migration to the area of injury.[12,13] Upregulated neurogenesis and neuronal progenitor migration has been repeatedly observed in the rodent, non-human primate, and human subventricular zone following focal ischemia as well as traumatic brain injury.[14,15] Unfortunately, this endogenous repair system is not sufficient to reverse the highly destructive effects of traumatic brain injury or stroke as evidenced by the substantial long term deficit experienced by survivors of these pathologies.

Discussion

Hyperacute & acute ischemic stroke pathogenesis

The brain has a significantly greater vulnerability to decreased perfusion than other organs due to its intrinsically high rate of metabolism required to maintain homeostasis within the cerebral parenchyma.[16] Cell death resulting from ischemic stroke tends to be heterogeneous due to differential tissue vascularization and occurs in hyperacute, acute, and chronic phases.[17,18]

Neurons located in tissue receiving less than 20% of normal perfusion undergo anoxic depolarization and energy failure in the seconds to minutes following occlusion, and subsequently undergo unprogrammed cell death, or necrosis, during the hyperacute phase of stroke.[19] This area of dead parenchyma is referred to as the infarct core.[8] The core of the stroke is highly cytotoxic due to cellular bursting and necrosing, ionic disregulation, and extensive Ca^{2+}/glutamate-mediated excitotoxicity.[20]

The acute phase of ischemic injury begins within minutes of vascular occlusion and lasts from hours to days after the ischemic event.[19] Parenchyma adjacent to the unsalvageable core is termed the peri-infarct zone or ischemic penumbra, and tissue within the penumbra receives sufficient collateral perfusion to temporarily maintain cellular viability.[8,21] Within minutes of vascular occlusion the decreased perfusion, as well as glutamate-mediated excitotoxicity leading to uncontrolled Ca^{2+} influx, increasing acidosis, ion gradient disruption, a potentiated free radical cascade, and tissue edema following blood brain barrier degradation increase metabolic demand placed on this tissue.[22-27] This results in markedly increased cellular mortality and expansion of the area of infarction into the previously viable penumbra.[28]

Acute traumatic brain injury pathogenesis

Traumatic brain injury initiates a similar pathology to that of ischemic stroke within the cerebral parenchyma in the minutes to hours following cranial insult.[7,29] As described above, glutamate-mediated excitotoxicity in response to localized dam-

age increased Ca^{2+} influx via *N*-methyl D-aspartate (NMDA) receptor activation increases the local metabolic demand and thus increases the area of injury.[30]

Common acute injury pathways

In the minutes following insult, damaged cells release high concentrations of glutamate into the surrounding tissue.[8] Even though glutamate is the major excitatory neurotransmitter in the brain, the release of this neurotransmitter in either stroke or traumatic brain injury is highly pathological as is it released in quantities much greater than normal.[20] Elevated glutamate levels result in prolonged and repetitive depolarization of neurons placing an increased metabolic demand on this tissue.[24] Postsynaptic depolarization is mediated in large part by excessive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptor activation in response to the elevated extracellular levels of glutamate.[31] Prolonged NMDA receptor activation as well as the subsequent opening of other voltage gated ion channels results in pathologically high intracellular Ca^{2+} levels.[32] Moreover, excitotoxic depolarization increases the energy requirement placed on surrounding parenchyma.[33]

Among many deregulated ionic concentrations, Ca^{2+} serves as an important mediator of glutamate release from the cell.[33] The primary pathologic mechanism of post-traumatic brain injury/stroke excitotoxicity is Ca^{2+} influx, and the intracellular Ca^{2+} overload upregulates numerous catabolic processes mediated by proteases, lipases and nucleases as well as other Ca^{2+} dependent pro-apoptotic enzymes.[24,34,35] ATP depletion is compensated for by anaerobic glycolysis which decreases pH within the surrounding tissue.[24] As the pH decreases, acid-sensing ion channels open and further potentiates Ca^{2+} influx, promoting the release of stored Ca^{2+} from the mitochondria and endoplasmic reticuli.[28,36] As cellular ATP levels are further depleted, the membrane bound $Na+/Ca^{2+}$ exchanger activity decreases and reduces the cell's ability to expel Ca^{2+}.[12,37,38] Thus, the cyclical potentiation of glutamate release and Ca^{2+} are major contributors to acute parenchymal insult.

Excitotoxicity-mediated Ca^{2+} overload in conjunction with anaerobic glycolysis and decreasing pH, damage the mitochondria of surrounding cells and enhance the production of free radicals. Free radicals are highly reactive molecules containing an unpaired electron, and are often referred to as reactive oxygen species (ROS).[39] Uncoupled oxidative phosphorylation via mitochondrial damage has been considered the primary source of reactive oxygen species, however recent studies indicate that superoxide, a highly potent reactive oxygen species, is generated predominantly by NADPH oxidase during excitotoxic NMDA receptor activation.[40,41] Additionally, systemically produced nitric oxide readily diffuses across the blood brain barrier, and reacts with superoxide and other reactive oxygen species yielding various types of highly reactive nitrogen.[42] Regardless of the proximal source, the free radical cascade within the local parenchyma greatly enhances cellular injury and promotes apoptosis in the minutes and hours following traumatic brain injury or stroke.[25,43]

Inflammatory pathogenesis and chronic injury

Cell death is greatly enhanced by inflammation, which contributes to the secondary injury, or the chronic phase of injury.[44] Edema resulting from neurovascular endothelial cell injury increases intracranial pressure, which then directly decreases cerebral perfusion and leukocyte-mediated inflammation function to exacerbate the initial injury as well as expand the lesion into the surrounding parenchyma.[19,44]

Neuroinflammation in response to acute injury is mediated by activated astrocytes as well as microglia, and is the prominent mediator of cell death and tissue injury in the days following a stroke or traumatic brain injury.[45] Microglia and astrocytes in and near the damaged parenchyma are activated, and subsequently release proinflammatory cytokines as well as chemokines in response to the initial injury.[12,13] In addition to recruiting astrocytes and microglia from surrounding tissue, activated astroglia degrade the blood brain barrier, increase tissue edema, and localize a highly destructive systemic inflammatory response to the site of injury in the hours and days following the initial insult.[26,46,47]

The cytokine and chemokine signals released by activated astrocytes facilitate systemic immune involvement by attracting circulating macrophages and leukocytes, as well as by promoting cell adhesion molecule upregulation in neurovascular endothelial cells.[48] Endothelial cells within the injured tissue also produce proinflammatory factors in response to injury, and enhance the adhesion of circulating immune constituents to the neurovasculature.[48] Matrix metalloproteinases are a category of protease upregulated by activated astrocytes which further degrade the blood-brain barrier and facilitate the influx of circulating immune constituents.[12,46] After extravasation and penetration of the *blood brain* barrier, these immune constituents release still more proinflammatory factors, greatly enhancing neuronal mortality, and markedly expanding the ischemic or traumatic lesion.[49,50]

Neuronal progenitors

Smart first demonstrated that the adult brain has the intrinsic ability to regenerate following injury.[51] Adult brains have endogenous stem cells in the subventricular zone that migrate to the olfactory bulb under normal physiological conditions, but can also aid in repair of damaged brain tissue.[51] Numerous studies have observed neurogenesis in adult mammals, and recent studies have demonstrated that there is a continuous production of neurons in adult non-human primates as well as in humans.[52-57] Of particular interest is the mammalian subventricular zone where a population of undifferentiated radial glial cells produce neural progenitors throughout adulthood.[58,59] Under physiological conditions, these neural progenitor cells migrate in a network of chains along the rostral migratory stream to the olfactory bulb where they differentiate into interneurons and functionally incorporate into existing neural circuitry.[60-63] These progenitors migrate from the subependymal zone of the lateral ventricle into the olfactory bulb in rats.[64]

The role of inflammation in progenitor differentiation & chemotaxic recruitment

Even though inflammation is the primary cause of neuronal damage in the hours to days following traumatic brain injury or stroke, inflammation can paradoxically play a role in neurogenesis, migration and regeneration in the weeks and months following injury.[29,30,65] Accordingly, numerous chemotaxic agents upregulated by tissue damage and inflammation can be classified as both pathologic and therapeutic depending on the time course and the extent of their expression.[66] Matrix metalloproteinase upregulation, for example, is highly detrimental in the acute phase of injury, however matrix metalloproteinases are upregulated by cells migrating from the subventricular zone to the damaged parenchyma.[67] Matrix metalloproteinases in the chronic phase of injury are involved in regeneration and repair of tissue damage.[68] Blocking matrix metalloproteinases in the weeks following injury significantly decreases neural progenitor migration and is highly detrimental to functional recovery.[26]

Numerous factors are capable of influ-

encing neural progenitor proliferation. In the days following focal ischemic injury, many of these factors are significantly upregulated during neural progenitor proliferation.[69-71] Proinflammatory factors, such as tumor necrosis factor-α (TNF-α) released by dying cells, upregulate progenitor migration to locations of neuroinflammation.[72] These factors enhance progenitor survival, and induce the migration of neuroblasts towards the site of ischemic or traumatic injury.[73,74]

In addition to promoting neurogenesis, chemotaxic factors released by damaged tissue appear to guide neural progenitors to the damaged parenchyma.[72,75,76] Moreover, chemotaxic factors released from the damaged tissue promote neurogenesis as well as angiogenesis, and neural progenitor migratory pathways to the damaged parenchyma tend to be closely associated cerebral vasculature.[77,78] Erythropoietin (EPO) and vascular endothelial growth factor (VEGF), for example, are commonly known to enhance angiogenesis, but also upregulate neurogenesis as well as direct neural progenitor migration.[15,28,78] Another highly versatile chemotaxic factor in this category is stromal cell-derived factor-1α (SDF-1α), which is upregulated by activated astrocytes and vascular endothelial cells following injury.[79-81]

Migrating neural progenitors require a physical scaffold to be able to physically move to the damaged parenchyma.[65] Under pathological conditions, cells migrate in association with reactive astrocytes as well as along cerebral vessels which they use as scaffolding.[82] Following stroke or traumatic brain injury, neuroblasts migrate in chains or individually to the damaged tissue.[83] The chains extended towards the striatum in close association with cerebral vessels.[77] Bovetti and colleagues observed that about half of the cells migrating to the adult olfactory bulb use blood vessels as a scaffold for migration.[84]

Post-injury progenitor migration: Time course and fate

In the days and weeks following ischemic stroke or traumatic brain injury, subventricular zone neural progenitor proliferation is greatly enhanced.[85] Migrating progenitor cells are diverted from the rostral migratory stream to the damaged parenchyma.[86] Moreover, the extent of injury has been demonstrated to determine the extent of subsequent neurogenesis and neural progenitor migration.[79,87]

Migration of newly differentiated neuroblasts begins within 24 hours of ischemic stroke or traumatic brain injury and these neuroblasts can be observed at the site of injury within one to two weeks.[88] Neural progenitor migration continues for several months following the initial traumatic/ischemic event.[86,89,90] The peak of number of cells migrating from the subventricular zone is observed approximately three weeks post injury and drops off in the weeks that follow.[91] However, the regenerative capacity of these stem cells is not intrinsically sufficient to repair the damage resulting from stroke or traumatic brain injury as most migrating neuroblasts die via apoptosis in the days and weeks following migration.[65]

Progenitor migration without a systemic immune response

Experimental data supports that initial suppression of the inflammatory response following stroke as well as traumatic brain injury is highly beneficial to functional outcome.[5] However, prolonged immunosuppression following traumatic or ischemic injury appears to be detrimental.[92] Tumor necrosis factor (TNF), for example, is a proinflammatory cytokine released by microglia as well as circulating immune constituents.[93] In the hours and days following injury, TNF promotes apoptosis, breakdown of the blood brain barrier, and inflammation.[94] Brain-injured TNF-deficient mice demonstrate improved outcome for the first week following brain injury.[92] However, TNF-deficient mice show diminished functional recovery in subsequent weeks, suggesting the effects of TNF may depend on the time course of its release.[92] Although the exact mechanism of TNF's temporal effects is not entirely understood, TNF has been demonstrated to upregulate production of neurotropic factor within reactive astrocytes as well as modulate neural progenitor differentiation.[44] Additionally, factors secreted by astrocytes and microglia such as monocyte chemotactic protein-1 (MCP-1) not only serve as chemoattractants for lymphocytes, basophiles and macrophages, but also neural progenitors. Knocking out MCP-1 expression significantly decreases neural progenitor migration *in vivo*.[95]

Although the initially destructive actions of the immune system in response to traumatic brain injury and stroke have been well characterized, the mechanism by which the immune response facilitates regeneration in the weeks following injury is not as well understood.[44] Recent studies have demonstrated that by suppressing the inflammatory response with progesterone for three to seven days following brain injury followed by tapered withdrawal of the immunosuppressant results in marked improvement in comparison to prolonged immunosuppression.[4] Improvements following short term immunosuppression were noted not only in functional outcome, but also neurogenesis in the subsequent weeks. These findings are consistent with negative outcomes following long-term administration of immunosuppressive corticosteroids to stroke patients.[96] Finally, progesterone-mediated immunosuppression does not appear as beneficial when abruptly halted rather then gradually discontinued.[4] Taken together, these results indicate that not only is the time course of inflammation important to functional outcome, but also the speed at which the inflammatory response commences. By gradually withdrawing immunosuppressants in the days following stroke, it is highly plausible that intrinsic pathways of immunomodulation are upregulated to mitigate the destructive effects, whereas such pathways cannot be upregulated rapidly enough to prevent substantial parenchymal injury and neuronal death in response to a rapid and robust immune response.

Conclusions

The neuroinflammatory response as well as the subsequent recruitment of a systemic immune response is highly similar following ischemic stroke or traumatic brain injury. Endogenous neuronal progenitor upregulation and migration occurs in the adult mammalian CNS and is also comparable in response to either pathology. A conceptual model of neuroinflammation as a continuum which is initially destructive but subsequently beneficial is supported by studies demonstrating that blocking neuroinflammation in the acute phase of injury greatly reduces functional deficit as well as neural damage, but inhibiting neuroinflammation in the chronic period tends to worsen functional outcome.[7] Therefore, management of both stroke and traumatic brain injury would likely benefit from the inhibition of inflammation in the hours and days following injury as well as from the tapered disinhibition of the inflammatory response in subsequent weeks.

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