

Stroke induced brain changes: implications for stem cell transplantation

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Ischemic stroke remains a major health problem associated with high mortality and severe morbidity. In spite of the extensive research in the field of stroke biology, there is little effective treatment for a completed disease onset. Numerous neuroprotective strategies have failed in clinical trials because of lack of efficacy or unacceptable side-effects. The challenge of clinical trial design is now to understand the process leading to ischemic brain injury and thus identify the targets for intervention in stroke. Therefore, uncovering cellular and molecular processes involved in ischemic brain injury is of critical importance. The review discusses the current understanding of these processes engaged in pathogenesis of stroke including excitotoxicity and inflammation. In addition recruitment of endogenous progenitors engaged in neurogenesis and vascular regeneration has been implicated. All of the aforementioned changes opted for therapeutic intervention to protect neurons in the region adjacent to the ischemic cerebral tissue and enhance cell recovery. Stem cell transplantation seems to offer a major promise of this therapy in stroke disorders.

Key words: brain ischemia, excitotoxicity, inflammation, neurogenesis, stroke

INTRODUCTION

Stroke is the third leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide (Luo 2010). Previously known medically as a cerebrovascular accident (CVA) is the rapidly developing loss of brain functions due to disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage (leakage of blood). Acute ischemic stroke accounts for about 85% of all cases while hemorrhagic stroke is responsible for almost 15% of all strokes (Sims and Mwyderman 2010).

The most common cause of stroke is the sudden occlusion of a blood vessel resulting in an almost immediate loss of oxygen and glucose to the cerebral tissue (Muir et al. 2007, Espinoza-Rojo et al. 2010). The deprivation of oxygen and glucose leads to several events including excitotoxicity, oxidative stress and

inflammation. It causes irreversible neuronal injury as well as oligodendrocytes, astrocytes and endothelial cells damage (Dirnagl et al. 1999, Lindvall and Kokaia, 2010). Moreover, stroke may also affect both white and grey matter and disrupt various anatomical pathways that need to be restored (Locatelli et al. 2008). Widespread tissue damage throughout the central nervous system (CNS) has been shown to cause marked and multifarious functional impairments in the ischemic brain. Deficits can include partial paralysis, difficulties with memory, thinking, language, and movements.

STROKE RISK FACTORS

Risk factors for stroke include those which cannot be modified, such as advanced age, previous stroke or transient ischemic attack (TIA), diabetes, atherosclerosis, atrial fibrillation, migraine with aura or thrombophilia (a tendency to thrombosis) and risk factors that are modifiable, and their correction reduces the chance of having a stroke as hypertension (high blood pressure), high cholesterol, cigarette smoking or psychosocial stress (Feigin 2005, Hankey 2006). These factors, which often coexist,

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have been estimated to account for 60% – 80% of stroke risk in the general population (Allen et al. 2008).

ISCHEMIC CASCADE AND EXCITOTOXICITY

Loss of cerebral blood flow rapidly limit the delivery of oxygen and glucose to neurons causing ATP reduction and energy depletion, initiating excitotoxic mechanisms that are deleterious for neuronal cells. These include activation of glutamate receptors and release of excess glutamate into extracellular space inducing neuron depolarization (Choi and Rothman 1990, Dirnagl et al. 1999, Lo et al. 2003). Over-activation of NMDA and AMPA receptors then leads to a generalized ionic imbalance within neurons, especially the increase of intracellular calcium. Massive calcium influx activates catabolic processes mediated by proteases, lipases, and nucleases (Ankarcrona et al. 1995). The calcium overload is then thought to activate multiple cell death pathways. Over-stimulation of glutamate receptors produces also other adverse effects i.e., compromising of organelle functions, increase of nitric oxide (NO) production and free radicals, persistent activation of proteases and kinases, increases in expression of pro-death transcription factors and immediate early genes (IEGs) which act as additional triggers of cell death (Lo et al. 2003, Semenov et al. 2008, Wang and Qin 2010). The notion that excitotoxicity leads exclusively to neuron necrosis has been abandoned as ultrastructural and biochemical analysis have been shown signs of apoptotic and autophagic cell death in ischemic neurons (Taoufik and Probert 2008). As both astrocytes and oligodendrocytes express NMDA and AMPA receptors, they are also vulnerable to high levels of glutamate and the excessive stimulation of glutamate receptors on glial cells induces excitotoxicity leading to cell death (Besancon et al. 2008).

INFLAMMATORY CELL RESPONSE IN CEREBRAL ISCHEMIA

The rapid loss of neuronal cells in the ischemic core and subsequent release of damage-associated molecular patterns (DAMPs) may contribute to the local inflammatory response of glia and endothelium in the brain. Inflammation is a critical mediator of brain damage in response to cerebral ischemia. As demonstrated both in animal models (Schilling et al. 2003,

Jablonska et al. 2010, Vandeputte et al. 2010) and in stroke patients (Gerhard et al. 2000) this post-ischemic inflammatory cascade involves a rapid activation of microglia and astrocytes.

Microglial cells play a critical role as resident immunocompetent and phagocytic cells in the CNS, forming a network of potential effector cells. They quickly and very sensitively respond to the changes in the brain, so the degree of microglial activation and cytokine expression upon ischemia can either be a contributor or the severity of injury (Nakajima and Kohsaka 2004). After stroke, the number of microglia is augmented in the lesion area by their proliferation (Janowski et al. 2008). Activated microglial cells are able to produce pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α , as well as other potential cytotoxic molecules including NO, ROS, and prostanooids. Several studies described a correlation between IL-1 β and IL-6 expression and ischemic injury (Denes et al. 2010). In contrast microglial TNF- α was found to protect neurons against ischemia as demonstrated in TNF-knockout mice (Lambertsen et al. 2009). Also the other factors released by microglia i.e. brain-derived neurotrophic factor (BDNF), insulin-like growth factor I (IGF-I) have been shown to be protective contributing to neurogenesis (Lucas et al. 2006, Ekdahl et al. 2009). Additionally, microglial cells could also have neuroprotective role mediated by neurotrophin release, glutamate uptake, and sequestering of neurotoxic substances (Koenigsknecht and Landreth 2004). Activated microglia may therefore have beneficial effects in the ischemic brain although this is still controversial and may depend on the type and the region-specific injury.

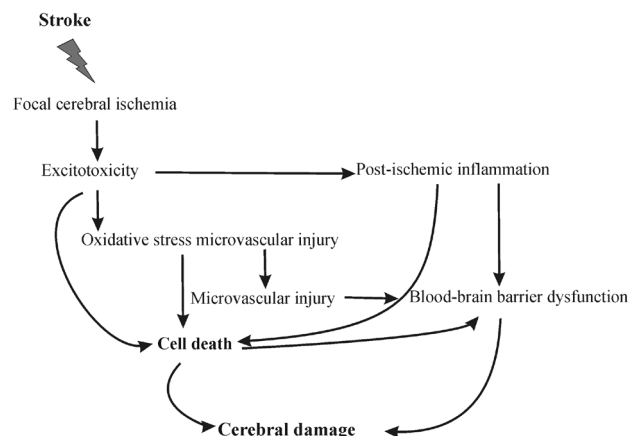


Fig. 1. Ischemic cascade leading to cerebral damage (Printed with the permission of publisher in accordance with BioMed Central's Open Access Charter, from Lakhani et al. 2009)

Recent data indicate that IL-1 β and TNF- α have been expressed by different subsets of microglia after ischemic stroke in mice (Clausen et al. 2008).

Astrocytes are considered to be less vulnerable to ischemic insults compared to neurons (Benavides et al. 2005). They respond to ischemia by production of inflammatory mediators including free radicals, cytokines, chemokines, proteases and vasoactive mediators (Markiewicz and Lukomska 2006, Thornton et al. 2008). Under these conditions astrocytes may compromise neuronal viability. However other functions of astrocytes likely improve neuronal survival during stroke. For example uptake of glutamate and release of neurotrophins enhances neuron viability during ischemia (Feeney and Stys 2009). Recent work also suggests that astrocytes beyond their influence on cell survival also contribute to angiogenesis, neuronal plasticity and functional recovery after stroke (Zhao and Rempe 2010).

Neurons even if they were acclaimed to play only passive role in the inflammation now are known to be involved in this process. Different products released by neurons i.e. neuropeptides or transmitters, as well as the membrane proteins CD22, CD47, CD200, CX3CL1 (fractalkine), intercellular adhesion molecule (ICAM-5), neural cell adhesion molecule (NCAM), semaphorins and C-type lectins have been shown to be engaged in inflammation (Tian et al. 2009). In addition, neurons can express low levels of major histocompatibility complex (MHC) molecules and actively promote T-cell apoptosis via the Fas–Fas ligand pathway (CD95–CD95L). Neuronal expression of the cannabinoid (CB1) receptor is implicated in suppressing inflammation. CB1 knockout mice more readily develop experimental autoimmune encephalomyelitis (EAE), the autoimmune model of multiple sclerosis (MS) (Pryce and Baker 2007). Neurons also favor the differentiation of T-regulatory cells, by providing a local microenvironment dominated by transforming growth factor- β 1 (TGF- β 1). The action of TGF- β 1 is important for the proliferative arrest of CD25⁺ TGF- β 1⁺ CTLA-4⁺ FoxP3⁺ T regulatory (Treg) cells. This autocrine effect may be the mechanism limiting the expansion of Treg cells in the CNS. Blocking the B7 and TGF- β 1 pathways prevents the CNS-specific generation of Treg cells developing unchecked T-cell proliferation (Liu et al. 2006). These findings show that generation of neuron-dependent Treg cells in the CNS is instrumental in regulating CNS inflammation.

It is known that neuronal cells are very susceptible to ischemia and damaged neurons are less able to maintain this protective shield, allowing further insults. So in the acute phase of stroke the first crucial line of defense is the activation of innate immune response, to opsonise and clear apoptotic cells. Then, innate immune response recruits cells of the adaptive immune system by secreting various cytokines and chemokines that induce adhesion molecules on the BBB, and by inducing the expression of costimulatory molecules on microglia. The innate response can be triggered through conserved pattern-recognition receptors (PRRs). One of this receptors are Toll-like receptors (TLRs), which bind highly conserved structural motifs either from pathogens (pathogen-associated molecular patterns, or PAMPs) or from damaged or stressed tissues (damage-associated molecular patterns, or DAMPs) (Liew et al. 2005). Some DAMPs, including heat shock proteins, uric acid, chromatin, adenosine and ATP, high mobility group box chromosomal protein 1 (HMGB-1), galectins and thioredoxin have adjuvant and pro-inflammatory activity. Other DAMPs include surfactant proteins A and D, hyaluronan, fibrinogen and aggregated, modified or misfolded proteins such as amyloid-beta (A β), α -synuclein and microtubule associated protein - tau. The other receptors for these endogenous stimuli of innate responses are unknown.

Endothelial cells, after their activation, similarly to glia, express inflammatory mediators including cytokines and chemokines. Also there is increase in expression of cell adhesion molecules including E-selectin, ICAM-1 and VCAM-1, which leads to the recruitment of peripheral blood cells/components, including platelets, neutrophils, macrophages and lymphocytes (Denes et al. 2010). After ischemia there is reduction of expression and stability of cerebrovascular tight junction proteins as occludins and claudins, which is often associated with an increase in the levels and activation of MMPs (Liu et al. 2009b) and can be exacerbated by systemic inflammatory conditions (McColl et al. 2008). Thus, cerebral endothelial activation leads to diminish in brain-blood-barrier (BBB) function.

Immune privilege in the CNS is partially dependent on the BBB, which is designed to limit the entry of solutes and ions into the CNS (Carson et al. 2006). Selective entry of compounds into the CNS takes place in the capillary venules. In contrast, cell migration takes place at the post-capillary venules, where cell migration is controlled by adhesion molecules,

cytokines and chemokines, and their receptors (Owens et al. 2008). In the regulation of properties of the BBB and immune response engaged are alike, microglia, astrocytes and neurons. Pro-inflammatory cytokines released by activated microglia and astrocytes cause blood-brain barrier disruption and further stimulate gliosis (Somera-Molina et al. 2009). Additionally, cytokines up-regulate the expression of cell adhesion molecules (CAMs), what help circulating leukocytes adhere to vessel walls and migrate into the brain with subsequent release of additional pro-inflammatory mediators (Huang et al. 2006).

There is also clinical and experimental evidence for peripheral inflammatory changes in response to cerebral ischemia. Leukocyte count increase in the peripheral blood is one of the earliest inflammatory responses to stroke identified in patients. Within few hours after ischemia cytokines up-regulate the expression of cell adhesion molecules (CAMs) what helps circulating leukocytes adhere to vessel walls and migrate into the brain with subsequent release of additional pro-inflammatory mediators and secondary injury in the penumbra. The first leukocytes recruited to the brain are neutrophils. These cells leads to secondary injury of potentially viable tissue by releasing pro-inflammatory cytokines and other cytotoxic products as proteases, ROS, MMPs (Wang and Qin 2007). Numerous studies show improved neurological outcome following neutrophil depletion and inhibition of adhesion molecules which facilitate neutrophil entry into injured brain, what confirm destructive role of this cells (Chopp et al. 1997). Increase in the levels of circulating monocytes is also observed but this tends to be delayed compared to the neutrophil response, being seen three days post stroke (Jablonska et al. 2010, Kochanek and Hallenbeck 2010).

The roles of lymphocytes are generally intended to play a negative role in ischemic brain pathogenesis. T- and B-cells have been identified by immunohistochemistry in post-ischemic brain and localized to infarction boundary zones, close to blood vessels (Jablonska et al. 2010, Stevens et al. 2002). The first information about the role of lymphocytes in reperfusion injury after ischemic stroke was based on experiment where blockade of migration through BBB results in reduced stroke volume as assessed 24 h after transient middle cerebral artery occlusion in rats (Becker et al. 2001). Hurn and coworkers presents that inducing stroke in severe combined immunodeficient (SCID) mice that lack T and B-cells might result in improved outcome for the brain relative to

immunologically intact mice (Hurn et al. 2007). These dates showed that lymphocytes play a detrimental role in acute stroke, but the mechanisms involved remained elusive. For these days we know that the major role of T-cells is production of proinflammatory cytokines such as IL-6, IL-1 β , TNF- α and TNF- β (Vogelgesang et al. 2010). Even less is known about a role for B lymphocytes in the brain inflammatory process.

INFLAMMATORY MEDIATORS IN CEREBRAL ISCHEMIA

As well as cellular changes in response to stroke, there are increases in levels of several secreted inflammatory mediators. In the experimental setting a large number of inflammatory molecules have been investigated in stroke. Among them pro-inflammatory cytokines have been suggested to contribute to the formation of ischemic damage, oedema and neuronal death. They are produced by immune cells as well as neural cells, including neurons and glia (Barone and Feuerstein 1999). Based on experimental stroke studies in rodents

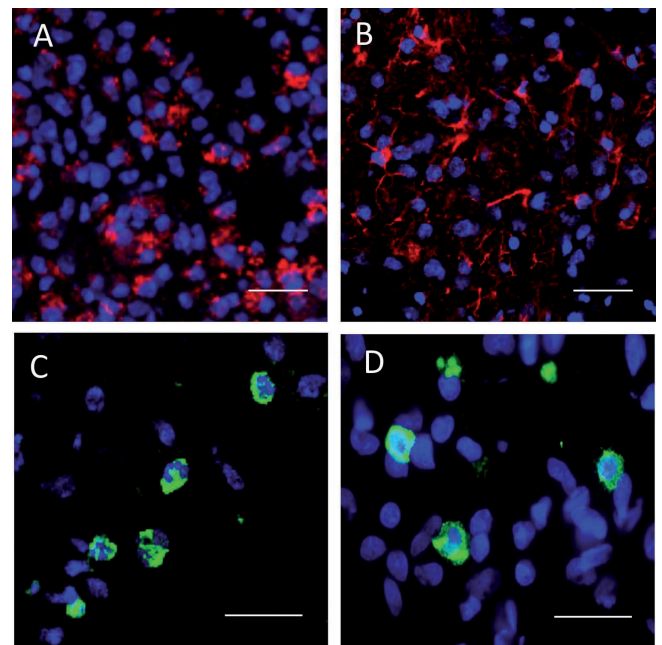


Fig. 2. Confocal analysis of the phenotypes of immune cells present in ouabain injured rat brain. (A) ED-1⁺ cells (macrophage/microglia) (red), (B) GFAP⁺ cells (astrocytes, red), (C) CD-15⁺ cells (neutrophils, green), (D) CD-5⁺ cells (T-lymphocytes, green) were visualized in injured rat brain area 72 hour after ouabain injection. Cell nuclei were counterstained with Hoechst 33252 (blue). Scale bar = 20 μ m (Jablonska unpublished data)

the reports demonstrated the expression of tumor necrosis factor- α (TNF- α), the interleukins (IL): IL-1 β , IL-6, IL-10, IL-20 and transforming growth factor (TGF- β 1).

One of the major pro-inflammatory cytokines is interleukin-1 (IL-1). Up-regulation mRNA of IL-1, especially IL-1 β (Rothwell 1999), has been shown within hours after stroke (Fan et al. 1995, Lu et al. 2005). The primary cells responsible for early expression of IL-1 seem to be microglia and perivascular macrophages. IL-1 can be also expressed by astrocytes, endothelial cells, and immune cells, and even there is some reports suggest that neurons can express IL-1. IL-1 plays a pivotal role in the induction of MMP-9 activity in acute and chronic inflammatory states (Ruhul Amin et al. 2003) leading to open the BBB by degrading tight junction proteins (Yang et al. 2007). Administration of exogenous IL-1 β intra-cerebroventricularly exacerbates ischemic injury whilst inhibition of endogenous IL-1 with IL-1 receptor antagonist (IL-1ra) protects against ischemic injury. Similarly, deletion of IL-1 β and IL-1ra results in markedly reduced ischemic damage and neuronal death (Banwell et al. 2009).

Tumor necrosis factor-alpha (TNF- α) is a crucial pro-inflammatory cytokine involved in response to the stroke. This pro-inflammatory cytokine is produced upon stimulation by monocytes, macrophages, T and B lymphocytes, neutrophils and mast cells. The cytokine exerts its biological effects via interaction with two high-affinity receptors R1 (p55) and R2 (p75). By R1 receptor TNF- α transduces death signals through FADD (Fas associated death domain) and by R2 activate the transcription factor NF- κ B. It has been shown that TNF- α have several important function in CNS. By its ability to cause increase in activation, proliferation and hypertrophy of microglia, TNF- α is able to regulate its own production (Kita et al. 1997). TNF- α also encourages the recruitment of leukocytes from peripheral circulation, regulate glutamatergic transmission and astrocyte activation (Pickering et al. 2005). Direct injection of TNF- α intracerebrally into mice, led to disruption of the blood brain barrier, as manifested by extravasation of dye, Evans blue, into the brain parenchyma. Correspondingly, experiments with using anti-TNF- α antibodies administered directly into the cerebroventricular system, reduced ischemic damage and improved functional outcome (Nawashiro et al. 1997). Apart from these data there are several studies

suggested neuroprotective character of TNF- α and its role in developing the tolerance to ischemic or traumatic brain injuries (Figiel, 2008, Liu et al. 2000). Preconditioning animals with TNF- α before ischemia showed remarkable tolerance to the insult, as evidenced by better functional outcome and lesion reduction (Liu et al. 2000). Alike studies on TNF- α null mutation mice showed acceleration of lesion and functional deficits (Gary et al. 1998).

Interleukin-6 (IL-6) is a multifunctional cytokine with various biological activities in immune regulation. It's mainly secreted by macrophages and T-cells and it exerts its effect by binding to the IL-6 specific membrane receptor (IL-6R) what leads to the activation of many downstream genes. IL-6 jointly with TNF- α or IL-1 is required for the induction of acute phase proteins many of which are protease inhibitors. It also has an anti-inflammatory role by inhibitory effects on TNF- α and IL-1, and activation of IL-1ra and IL-10. IL-6 is critically required to control the extent of local or systemic acute inflammatory response, particularly the level of pro-inflammatory cytokines in the local and systemic compartments, respectively (Xing et al. 1998). IL-6 is up-regulated following cerebral ischemia. Its increasing level in serum and CSF in stroke patients correlated with infarct volume and early worsening of neurological score (Vila et al. 2000).

Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is a cytokine produced by a variety of cells, including monocytes/macrophages, T-cells, B-cells, and mast cells. In CNS the main source of IL-10 are microglia and astrocytes (Williams et al. 1996). Expression of IL-10 is elevated during the course of most major diseases in the CNS and promotes survival of neurons and glial cells in the brain by blocking the effects of pro-apoptotic cytokines and by promoting expression of cell survival signals (Spera et al. 1998). IL-10 is an important anti-inflammatory cytokine. It is capable of inhibiting synthesis of pro-inflammatory cytokines like IFN- γ , IL-2, IL-3, TNF- α and GM-CSF made by cells such as macrophages, microglia and regulatory T-cells (Koch et al. 1997, de Waal Malefyt et al. 1991). It down-regulates the expression of MHC class II antigens and costimulatory molecules on macrophages. Interleukine-10 also enhances B-cell survival, proliferation, and antibody production. Stimulation of IL-10 receptors regulates numerous life- or death-signaling

pathways—including Jak1/Stat3, PI 3-kinase, MAPK, SOCS, and NF-kappa B, promoting cell survival by inhibiting ligand- and mitochondrial-induced apoptotic pathways. Also, IL-10 induces inactivation or unresponsiveness of brain-infiltrating T-cells by inhibiting cell signaling through the costimulatory CD28-CD80/86 pathway (Akdis et al. 1998). It was shown that after permanent focal ischemia in rats administration of adenoviral vectors encoding human IL-10 reduced infarct volume (Ooboshi et al. 2005) and IL-10 knockout mice showed larger infarct volume than the wild-type (Grilli et al. 2000). In patient with stroke low serum level of IL-10 is associated with poor outcome and neurological worsening (Vila et al. 2003).

ENDOGENOUS NEUROGENESIS AFTER CEREBRAL ISCHEMIA

Acute injuries to CNS such as stroke induce neural progenitor proliferation in adult brain which might be an endogenous attempt to self-repair. Although ischemia induces cell death in the core of the infarct, the progenitor cells residing in the subventricular zone (SVZ) and the dentate gyrus (DG; both regions that are remote from the infarct) proliferate and migrate to the site damage (Arvidsson et al. 2002, Yagita et al. 2001). The number of proliferating cells in the ipsilateral SVZ was reported to be significantly increased by 2 – 14 days following ischemic onset in rats with a peak at 7 days (Zhang et al. 2001). Similarly, Liu and coauthors demonstrated a 12-fold increase in cell proliferation in the DG at 1–2 weeks after a global ischemia in adult gerbils (Liu et al. 1998). Many other studies also confirmed that focal or global cerebral ischemia potently stimulates neurogenesis in the DG of adult rodents and monkeys (Kawai et al. 2004, Koketsu et al. 2006). Strikingly, stroke induced neurogenesis has recently been observed in the adult human brain, even among the elderly (Minger et al. 2007). In addition to the well-established neurogenic regions, cerebral ischemia was reported to promote neurogenesis in the injured cerebral cortex (Shin et al. 2008). In addition to inducing neural progenitor cell proliferation in brain, cerebral ischemia also promotes recruitment and mobilization of stem cells from the bone marrow to brain (Liu et al. 2009).

Cerebral ischemia-induced neurogenesis might be an adaptive, compensatory process that is regulated by many various factors. Several studies have demon-

strated that growth factors play an important role following ischemia-induced brain damage by enhancing the survival and stimulating the proliferation of endogenous neural progenitor cells (Dempsey and Kalluri 2007, Kalluri and Dempsey 2008). The increase in ischemia induced neurogenesis could therefore be due to the up regulation of growth and trophic factor content. A plethora of neuronal mediators were shown after brain injury. Of importance are GDNF, BDNF, FGF2, IGF1 and VEGF (Watanabe et al. 2004, Baldauf and Reymann 2005, Tureyen et al. 2005, Schäbitz et al. 2007). These factors seem to modulate various steps of the post-ischemic neurogenesis. However, not all growth factors are stimulatory in function; some are stimulators of neural progenitor proliferation, whereas others block the self-renewal of cells. For example, TGF- β 1 can directly block cell proliferation by up-regulating the expression of cell cycle inhibitors (Wachs et al. 2006).

Recent studies have shown that ischemia induces the migration of neuroblasts into the tissue adjacent to the infarct (Thored et al. 2006, Janowski et al. 2008). In the adult brain, migrating neuroblasts can replace damaged neurons after severe traumatic brain injury (TBI). Little is known about which factors determine the neurogenesis after TBI but there are some evidence that the nerve growth factor (NGF) and doublecortin (DCX) can influence neurogenesis and neuronal repair (Chiaretti et al. 2008). NGF leads to positive trophic effects inducing the up-regulation of nicotinic receptors in the brain, improving significantly the cortical blood flow and counteracting the posttraumatic cerebral ischemia (Murdoch et al. 1998). Experimental studies have shown that increased level of induced neurogenesis with a marked increase in the number of DCX-immunoreactive migrating neuroblasts (Wilde et al. 2000). The NFG and DCX up-regulation correlated with improved outcome probably because both these factors activate the neuroprotective mechanisms of TBI. The observed changes of NGF and DCX expression may reflect an endogenous attempt of neuroprotection against the biochemical and molecular cascades triggered by traumatic insult (Koizumi et al. 2006).

The matrix metalloproteinases (MMPs) seem to be involved in the migration of new cells derived from neural precursors, and proteolytic processing of ECM proteins by MMPs was demonstrated to regulate the migration of cells, perhaps by clearing the path for them (Wojcik et al. 2009). In addition, chemotactic

signals like SDF-1 (stromal cell-derived factor 1) and its receptor CXCR-4 (CXC chemokine receptor 4) have been demonstrated to play a role in the directional migration of neuroblasts in CNS towards an infarcted area (Imitola et al. 2004)

Although in response to ischemia, several immature DCX-positive neuronal cells were shown to be migrating towards striatum and cortex, the factors responsible for promoting neuroblast differentiation are unknown. Hence it is speculative, TGF- β 1, which is up-regulated after ischemia, may be responsible for

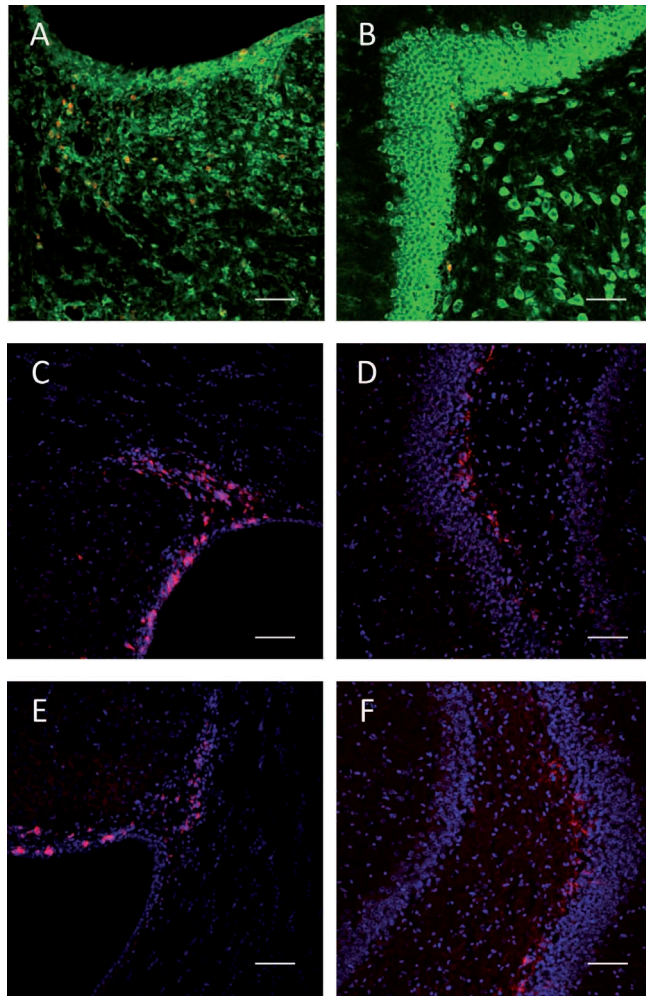


Fig. 3. Coronal sections of ouabain injured rat brain depicting neurogenesis in SVZ (A,C,E) and SGZ (B,D,F) regions. Proliferating (BrdU⁺) cells (red) accompany with high activity of MMPs (green) were observed in SVZ (A) and SGZ (B) 72 hour after ouabain injection. Concomitantly migrating neuroblasts (DCX⁺) and (PSA-NCAM⁺, red) were seen in SVZ (C, E respectively) and SGZ (D, F, respectively). Cell nuclei were counterstained with Hoechst 33252 (blue). Scale bar = 20 μ m (Jablonska unpublished data)

neuronal differentiation during the post-ischemic neurogenesis (Kalluri and Dempsey 2008). Nevertheless, some growth factors like bone morphogenetic protein-4 (BMP-4) can promote the differentiation of neural stem cells by activating proteins (such as Smads), which are translocated into the nucleus to induce gene expression (Fukuda et al. 2007).

The final outcome of stroke depends on the spatial and temporal expression of different factors following ischemia. Vascular supply in the infarct area is crucial to support neurogenesis. In the ischemic border, angiogenesis involving vascular sprouting from mature endothelial cells of pre-existing blood vessels creates a hospitable microenvironment for neuronal plasticity, leading to functional recovery (Locatelli et al. 2008). Interestingly, acute cerebral ischemia in human individuals leads spontaneously to a threefold increase in hematopoietic stem cell (CD34-positive) count in the peripheral blood (Hass et al. 2005). These cells may themselves show limited incorporation but serve to stimulate angiogenesis by secreting numerous angiogenic factors including hepatocyte growth factor (HGF), VEGF and IGF-1 (Majka et al. 2001)

Such a rich vascular environment along with generation of neuronal mediators by CD34-positive cells i.e., VEGF, FGF-2 and IGF-1 enhances neuronal regeneration (Jin et al. 2002). Thus, angiogenesis and neurogenesis seem to be coupled processes in the brain after stroke (Teng et al. 2008). In addition, cerebral endothelial cells, activated by ischemia, increase neural progenitor cell (NPC) proliferation and neuronal differentiation, as demonstrated in vivo (Zhang et al. 2007).

Interestingly, the stroke damaged adult brain preserves some replacement capacity to repair itself. This spontaneous recovery depends on brain's plasticity in terms of replacement of afferent and efferent connections and synaptogenesis. Hence, neuronal plasticity and reorganization of neural circuitries contribute to spontaneous recovery to varying degrees leaving patients with persistent motor, sensory and cognitive impairments. Apart from current handling strategies for stroke primarily focus on reducing the size of ischemic damage there is no effective treatment to promote recovery.

Recently, stem cell-based approaches have received much hope as potential methods used therapeutically. The findings reported from preclinical and clinical studies demonstrate that stem cell-based therapies have the potential to improve the clinical outcome in

stroke patients (Bühnemann et al. 2006, Bliss et al. 2009, Hicks and Jolkkonen 2009, Lee et al. 2010). Various possible mechanisms of action of the transplanted cells have been proposed. An attraction of exogenous cells is their potential to replace lost circuits; however evidence for this is limited. It is likely and perhaps more importantly is highlight potential side effects i.e., neuroprotection, immunomodulation, enhancing endogenous repair processes, vascular regeneration and induction of host brain plasticity. This effect can be elicited by grafted cells and their derivatives or released trophic factors and mediators. However, many questions remain to be answered before clinical applications of cell therapy for stroke in safe and effective manner.

CONCLUSIONS

Ischemic brain injury is a complex pathology that not only leads to cell death through energy depletion but also triggers a variety of post-ischemic responses including inflammation. Although ischemia induces cell death in the core of infarct, the progenitor cells residing in neurogenic regions of the brain proliferate and migrate following ischemic insult. Postischemic endogenous neurogenesis has been recognized as a compensatory mechanism to repair the damaged tissue. However, the intrinsic regeneration of the brain after ischemic injury is not efficient. Stem cell transplantation offers a promising new therapeutic strategy for stroke not only to prevent damage, which has been focus of conventional therapy, but also to actually repair the injured brain. Despite the fact that cell transplantation for stroke is still in a nascent stage and the experimental data are too preliminary and insufficient to assess its efficacy in the clinical trials, early work suggests that cell-based therapy may be adapted to use in ischemic stroke patients.

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