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Molecular Imaging of Stem Cell Therapy for Cerebral Ischemia in the Aged Brain

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ABSTRACT

Cell therapy has emerged as a promising approach to improve recovery after stroke. However, progress toward the development of efficient cell based therapies for ischemic stroke has been so far disappointing. The main problem remains the poor understanding of the dynamics of cellular interactions between host cells and administered cells for therapeutic purposes. The pathophysiological evolution of stroke events is driven by complex cellular interactions between many different cell types whose sequential recruitments have been insufficiently documented due to the lack of non-invasive imaging modalities. Specifically, the interplay between host neuroinflammation, which is considered to be a major obstacle to exogenous-mediated neuronal precursor cells, and exogenously administered stem cells, remains virtually unknown. Phagocytosis of dead and dying neurons and neuronal debris is beneficial in part because it reduces inflammation. However, microglia can also phagocytose live neurons, live neuronal progenitors or live stressed-but-viable neurons like those presumably occurring after transplantation causing death of the engulfed cell and compromising cell therapy using neuronal precursor cells. This lack of basic knowledge critically limits the optimization of timing and route of administration of stem cells for therapeutic purpose. In this review, we aim to decipherthe dynamic interplay between host neuroinflammation and therapeutic stem cells for regeneration after stroke by two non-invasive molecular imaging approaches (two photon microscopy and magnetic resonance imaging at high magnetic field) and their impact on behavioural outcome measures.

Keywords: Aging, Cell therapy, Imaging, Two photon microscopy, Magnetic resonance imaging

INTRODUCTION

Stroke is the third most common cause of death and the leading cause of disabilities worldwide. The damages after stroke generally have two different causes, the primary and secondary insult. The primary insult is mediated by the ischemic event itself and leads to oxygen and glucose deprivation, crucial substrates for the survival of the brain tissue, whereas the secondary insult is caused by the inflammatory response after stroke and mainly affects the penumbra, a brain area with a slightly compromised blood-supply that surrounds the ischemic core. Up to date, tPA (tissue plasminogen activator) is the only FDA approved therapeutic treatment for ischemic stroke [1]. But due to severe side effects and because of its very limited time window it is only applicable to less than 10 % of all stroke patients [1], thus indicating an urgent need for alternative treatments.

Up to now, studies of many brain diseases have limited their

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attention on the focal lesion, ignoring the wider impact of the directly affected territory on other brain regions. In this review we approach the brain as a rather horizontally organized, complex network where focal lesions will have far ranging effects on distal brain regions and their corresponding functions. This approach provides new insight into the mechanisms and reasons underlying functional disturbances and opens new avenues for future diagnosis. Specifically, through this novel approach we expect to make predictions with regard of the efficacy of stem cell therapy of ischemic stroke by gaining insight into of the time window of application and route of administration of stem cells on a background of neuroinflammation in aged subjects and to develop an experimental, pre-clinical rationale for the development of cell based therapeutic strategies for the treatment of stroke and neurodegenerative diseases.

Cell Therapy has Emerged as a Promising Approach to Improve Recovery after Stroke

However, progress toward the development of efficient cell based therapies for ischemic stroke has been so far disappointing. The main problem remains the poor understanding of the dynamics of cellular interactions between host cells and administered cells for therapeutic purposes. The pathophysiological evolution of stroke events is driven by complex cellular interactions between many different cell types whose sequential recruitments have been insufficiently documented due to the lack of non-invasive imaging modalities. Specifically, the interplay between host neuroinflammation, which is considered to be a major obstacle to exogenous-mediated neuronal precursor cells, and exogenously administered stem cells, remains virtually unknown.

Sub toxic insults such as inflammation, stressed but viable neurons may reversibly expose, eat-me signals, may expose the “eat-me” signal phosphatidylserine (PS) on neuronal surface. Activated microglia detect exposed ‘eat-me’ signals and engulfment of neurons or parts of neurons exposing such signals follows. This process has been coined primary phagocytosis or “phagoptosis” [2,3]. Toxic neuronal insults, such as dying neurons after stroke, irreversibly expose the “eat-me” signal recognized by primed microglia resulting in the phagocytosis if dead neurons or the so-called secondary phagocytosis [4]. There are “eat me” signals other than phosphatidylserine. During brain development and normal functioning in the adult, immune molecules, including complement proteins, C1q and C3, have emerged as critical mediators of synaptic refinement and plasticity via C3-dependent microglial phagocytosis of synapses. Following an acute injury such as stroke the apoptotic neurons release a chemotactic signal such as fractalkine/CX3CL1 [5,6] and microglia expressing the fractalkine receptor(CX3CR1), promotes phagocytosis of apoptotic cells expressing CX3CL1 [5,7]. The complement components C1q and C3, which are produced by microglia and astrocytes, may induce phagocytosis by binding opsonized/altered neuronal surfaces. In this process C1q promotes the conversion of C3, expressed by microglia, to C3b. C3b then opsonizes neurons and is recognized through complement receptor 3 (CR3) expressed by activated microglia.

Phagocytosis of dead and dying neurons and neuronal debris is beneficial in part because it reduces inflammation. However, microglia can also phagocytose live neurons, live neuronal progenitors or live stressed-but-viable neurons like those presumably occurring after transplantation causing death of the engulfed cell and compromising cell therapy using neuronal precursor cells.

Mild neuroinflammation can be beneficial for regenerative events aimed at functional restoration after stroke [8]. Cell therapy itself can be used during the first week post-stroke to limit neuroinflammation in animal models [9-11]. However, persistent post-stroke neuroinflammation results in decreased proliferation of the newly born NPCs and ineffective integration into the circuitry of the re-organized brain area [12]. Moreover, a number of studies have demonstrated that neuroinflammatory processes can induce apoptosis in NPCs and immature neurons [13,14] and decrease the efficacy of both stroke-induced neurogenesis and exogenously supported neurogenesis. This hypothesis is supported by findings from studies using anti-inflammatory drugs such as indomethacin or minocyclin block microglia-induced apoptosis of NPCs in a pro-inflammatory milieu [15-17]. More recently studies directly implicated TNFa produced by lipopolysaccharide-activated microglia is as a key determinant in microglia induced-apoptosis in mouse NPCs in vitro and in vivo [18].

This lack of basic knowledge critically limits the optimization of timing and route of administration of stem cells for therapeutic purpose. In the present review, we aim to investigate the dynamic interplay between host neuroinflammation and therapeutic stem cells for regeneration after stroke by combining non-invasive molecular imaging modalities (two photon microscopy (2P-LSM) and magnetic resonance imaging (MRI) at high magnetic field) with in vivo neurophysiological and behavioural outcome measures. Up to now, studies of many brain diseases have limited their attention on the focal lesion, ignoring the wider impact of the directly affected territory on other brain regions. In the proposed project we approach the brain as a rather horizontally organized, complex network where focal lesions will have far ranging effects on distal brain regions and their corresponding functions. This approach provides new insight into the mechanisms and reasons underlying functional disturbances and opens new avenues for future diagnosis. Specifically, through this novel approach we expect to make significant improvements in the efficacy of stem cell therapy of ischemic stroke by optimizing the time window of application and route of administration of stem cells on a

background of neuroinflammation in aged subjects. Finally, we expect to decipher experimental, pre-clinical rationale for the development of cell based therapeutic strategies for the treatment of stroke and neurodegenerative diseases.

Role of the Immune Response on Tissue Protection and Recovery

We have explored the role of the polarization phenotype of microglia and macrophages for the neuronal survival and functional deficit, functional improvement respectively. Injecting the microRNA 124 (mir-124), we found a substantial shift from the pro- to the anti-inflammatory phenotype, combined with a significantly higher neuronal survival. In parallel, there was a tight correlation between decrease of neurological deficit and increase of anti-inflammatory (M2) phenotype [19,20].

Stem Cell Therapies in Preclinical Models of Stroke Associated with Aging

Stroke has limited treatment options, demanding a vigorous search for new therapeutic strategies. Initial enthusiasm to stimulate restorative processes in the ischemic brain by means of cell-based therapies has meanwhile converted into a more balanced view recognizing impediments related to unfavorable environments that are in part related to aging processes. Since stroke afflicts mostly the elderly, it is highly desirable and clinically important to test the efficacy of cell therapies in aged brain microenvironments. Although widely believed to be refractory to regeneration, recent studies done by us using both neural precursor cells and bone marrow-derived mesenchymal stem cells for stroke therapy suggest that the aged rat brain is not refractory to cell-based therapy, and that it also supports plasticity and remodeling [21,22]. Yet, important differences exist in the aged compared with young brain, i.e., the accelerated progression of ischemic injury to brain infarction, the reduced rate of endogenous neurogenesis and the delayed initiation of neurological recovery. Pitfalls in the development of cell-based therapies may also be related to age-associated comorbidities, e.g., diabetes or hyperlipidemia, which may result in maladaptive or compromised brain remodeling, respectively. These age-related aspects should be carefully considered in the clinical translation of restorative therapies [23]. In the last 3 years, upon acquiring a 2P-LSM microscope, now the gold standard of microglia reaction [24-26], our focus has been on in vivo monitoring of the efficacy of anti-inflammatory therapies by quantifying microglia reaction to focal (**Figure 1)** or global lesions [27].

Functional Dynamics of Stem Cell Grafts

For almost 20 years, our research has focused on stem cell based regeneration of cerebral disorders. Based on the long-standing extensive work on stroke pathophysiology we have concentrated on various aspects of stem cell dynamics such as migration, proliferation, vitality, and neuronal differentiation. Using high resolution high field in vivo MRI at 7Tesla, we were the first to monitor directed stem cell migration towards the ischemic target in noninvasive longitudinal imaging studies [28]. Optimization of the bioluminescence imaging (BLI) for brain studies and identification of maximally sensitive luciferases allowed the in vivo assessment of stem cell graft vitality during longitudinal investigations [29,30]. Combination of this BLI protocol with 19F-MRI solved the controversial debate over the best suited location for graft vitality: it has been shown that the vitality of grafts in the peri-infarct area is equivalent to that of grafts in healthy tissue, thus allowing to safely implant stem cells close to but outside of the ischemic target zone [31]. From further studies, it was concluded that the graft size, but not the immune response to the grafting, influences the vitality. This resulted in recommendation of maximal graft size [32-34].

Differentiation and Integration of Neural Stem Cell Grafts

In a major project the temporal differentiation profile of human neural stem cells (NSCs) after grafting was identified. Stem cells were transduced to express imaging reporters under cell-specific control for the in vivo monitoring of selected stages of differentiation. For this purpose, luciferases for detection by BLI and fluorescent markers for immunohistological validation of in vivo observations by immunohistochemistry were chosen as imaging reporters. Setting the imaging reporters under gene control of early (Doublecortin) and late (Synapsin) neuronal differentiation, a time line was generated which permits for the first time the direct correlation between functional improvement, assessed by behavioral test, and the neuronal differentiation of the graft and its capacity to integrate. This time line further allows a reliable discrimination between discussed modes of action of stem cells for a functional improvement: bystander effect (during early phase before neuronal differentiation) and tissue replacement (after neuronal differentiation and during cell integration) [32]. In a further study the graft innervation by the host tissue was unraveled. Whole-brain light sheet microscopy of a translucent brain permitted at cellular resolution the afferent connection from far-ranging neuronal sites to the graft. The corresponding anatomical assignment of the connecting host tissue regions was achieved by coregistration of the whole-brain light sheet data set with high-resolution anatomical MRI and mouse brain atlas [35].

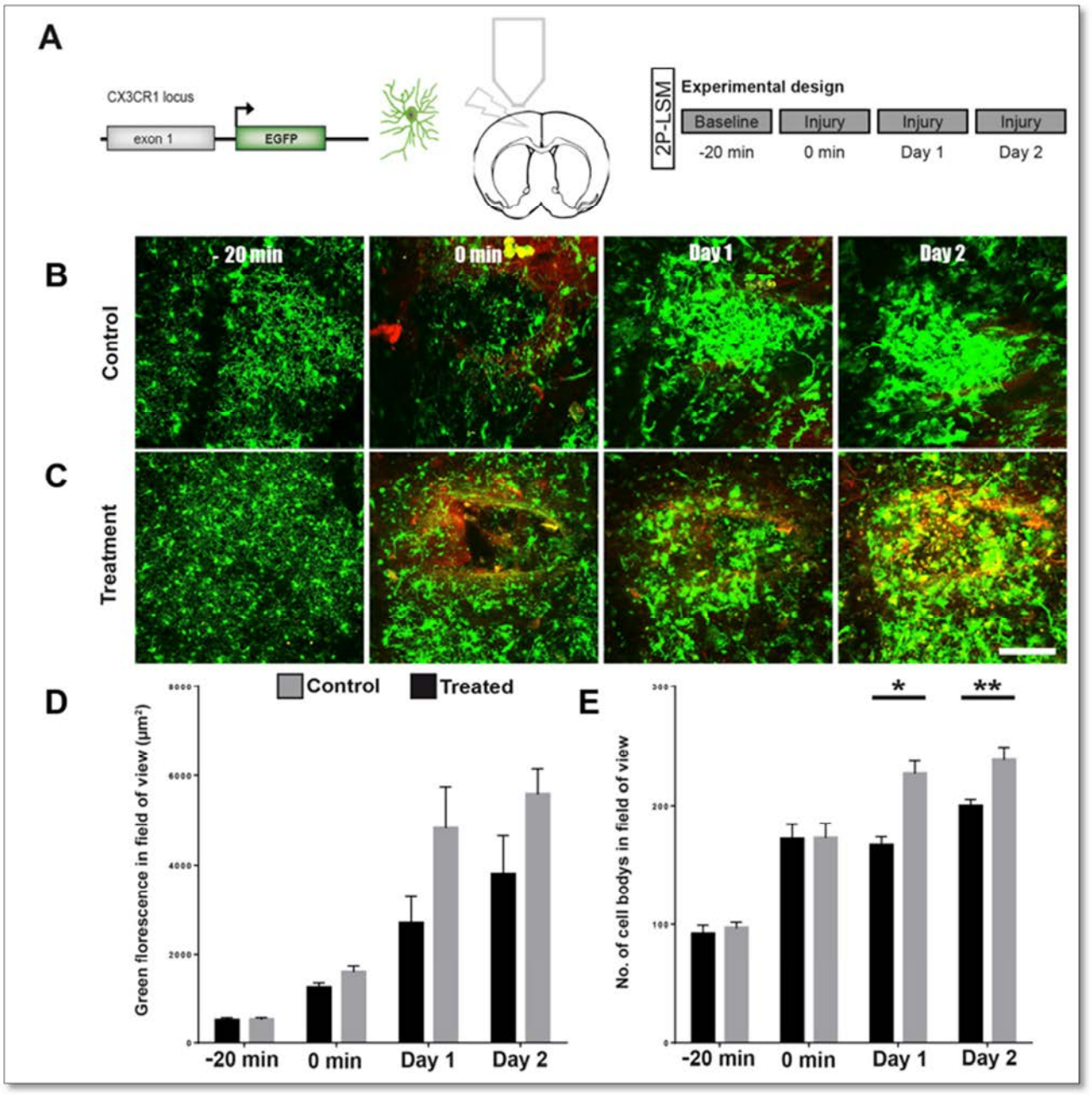

Figure 1. Two-Photon microscopy time laps images of an anesthetizes mouse right somatosensory cortex, taken over a period of three days(A)show green microglia of a transgenic mice (EGFP was bonded to exon1 of the CX3CR1 locus[7]), (B, C) reacting to a 2 mm deep stab wound injury done using a 30G needle. Every experiment has a 20 min baseline (-20 min) for every group investigated . After this baseline, the injury is done, and with the same needle a 1 µl red solution, obtained by adding Sulfurodamine B to vehicleis directly injected in the cortex. (D).Quantification of the number of microglia over time, after the lesion, shows a delay microglia proliferation in the treated animals compared with the controls. (Mean±SEM, *<0.05, **p<0.01).

Neuronal Networks and Post-Stroke Recovery

The understanding of cerebral diseases has moved from the focal attention on the lesion itself to more global concepts of far ranging effects in neuronal networks during the past years. Invasive approaches of experimental investigations are either limited to a few local recordings such as electrophysiology or permit only a description of a single time point because of invasiveness of the method. Modern noninvasive molecular imaging modalities not only allow

the investigation of dynamic cellular processes (see above) but have recently begun to open new doors to generate temporal profiles of dynamic processes relating to both structural and functional connectivity networks in the brain. Animal studies in rodent models can now be investigated with impressive sensitivity and resolution, thus unravelling mechanisms and interactions between various components such as different cell types during lesion development and (therapeutically intervened) outcome. Thus, investigations by Dijkhuizen and colleagues have shown the disturbance of the functional networks after stroke induction, affecting the ischemic hemisphere but also extending the deficits into the transhemispheric connections of homotopic areas of the motor and sensory cortex [36,37].

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