Imaging neuroinflammation after brain injuries by ultrasensitive MRI and two-photon laser-scanning microscopy

ANJA SCHELLER\textsuperscript{1)}, DENIS VIVIEN\textsuperscript{2)*}, FRANK KIRCHHOFF\textsuperscript{L,3)*}, CYRILLE ORSET\textsuperscript{2)*}, RALUCA ELENA SANDU\textsuperscript{3)}, AUREL POPA-WAGNER\textsuperscript{3,4)*}

\textsuperscript{1)Department of Molecular Physiology, University of Saarland, Germany}
\textsuperscript{2)University of Caen Basse Normandie, UMR-S INSERM U919, GIP CYCERON, Caen Cedex, France}
\textsuperscript{3)Department Functional Sciences, University of Medicine and Pharmacy of Craiova, Romania}
\textsuperscript{4)Department of Psychiatry, University of Medicine Rostock, Germany}

*These authors have contributed equally to the manuscript.

Abstract
Worldwide, stroke is the leading cause of disability in the aging population. Neuroinflammation is a common feature of acute stroke and is considered to be a major obstacle to endogenous neurogenesis and exogenously administered stem cells. Therefore, drug and cell therapies aimed at suppressing post-stroke inflammation have 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 disappointing mainly because the interplay between host neuroinflammation and stem cell-based therapies during the acute stroke and the recuperation phase is virtually unknown. The pathophysiological evolution of stroke events indeed seems driven by complex cellular interactions between several different cell types whose sequential recruitments have been insufficiently documented due to the lack of respective technologies, in particular, of non-invasive imaging modalities. The development of \textit{in vivo} ultrasensitive magnetic resonance imaging (MRI) and two-photon laser-scanning microscopy has revolutionized our understanding of neuroinflammation. Therefore, the purpose of this review is to highlight the interplay between host neuroinflammation, which is considered to be a major obstacle to exogenous-mediated neuronal precursor cells, and exogenously administered stem cells.

Keywords: stroke, neuroinflammation, 2P imaging, ultrasensitive magnetic resonance imaging.

\section*{Introduction}
Worldwide, stroke represents a major problem of public health. According to epidemiological studies, it is estimated that stroke currently accounts for more than five million death worldwide, equivalents to about one tenth of all deaths. Two-thirds of these deaths occur in patients living in developing countries and 40\% of the subjects were aged less than 70 years. More data show that cerebrovascular disease is the leading cause of disability in adults and each year millions of stroke survivors have to adapt to a life with restrictions in activities of daily living because of stroke. Romania has a rapidly aging population and one of the highest incidences of stroke in Europe, ranking third after Russia and Bulgaria and only little is known about the causes and consequences of stroke in this population. Furthermore, Romania lacks a significant research effort on the problem.

Apart from the use of tissue plasminogen activator as a fibrinolytic (Actilyse\textsuperscript{8}) in eligible patients, no treatment exists to promote neuroprotection or neurorepair. Despite the approved indication of systemic thrombolytic therapy for the acute management of ischemic stroke, its use is limited given a strict eligibility criteria and a risk for hemorrhagic transformation as a feared adverse effect. Thus, cell therapy has emerged as a new, promising approach to improve recovery after stroke. However, progress toward the development of efficient cell based therapies for ischemic stroke has been disappointing mainly because the experimental design is static and lacks the dynamics of cellular interactions between host cells and administered cells for therapeutic purposes. The pathophysiological evolution of stroke events indeed seems 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. The interplay between host neuroinflammation, which is considered to be a major obstacle to exogenous-mediated neuronal precursor cells, and exogenously administered stem cells is virtually unknown. Similarly, the optimal timing and route of administration of stem cells are unknown.

Clinical studies aimed at reducing the post-stroke inflammation have been largely disappointing as has been the anti-cell therapy that has emerged as a promising approach to improve recovery after stroke mainly because the experimental design is static and does not take into account the timecourse of the dynamics of cellular interactions between host cells and administered cells for therapeutic purposes. However, the development of \textit{in vivo} ultrasensitive MRI and two-photon laser-scanning microscopy (2P-LSM) has allowed the study microgli
functions under steady state in intact brains of living animals. In particular, in vivo imaging has revolutionized our understanding of neuroinflammation after damages to the brain [1, 2] and has paved the way for a deeper understanding of the complex interaction between post-stroke neuronflammation and cell therapy.

Glial cells in the brain

Microglia cells are highly dynamic cells with motile processes, which are controlling the direct microenvironment constantly for microlesions, etc. [1, 3, 4]. ATP and subsequently NO modulate the activation of microglia after injury [1, 5]. Furthermore, capillary blood flow adjacent to microglial somata determines the dynamics of microglial processes in ischemic conditions [6]. In the CNS, the fractalkine receptor CX3CR1 is only expressed by microglial cells, and was therefore used to specifically label microglia by generating a transgenic animal with insertion of the green fluorescent protein EGFP into the receptor locus. In homozygous EGFP mutant mice, it is possible to study the knock out of the fractalkine protein EGFP into the receptor locus. In homozygous EGFP mutant mice, it is possible to study the knock out of the fractalkine receptor leads to significantly smaller infarct volumes and of microglial cells. The knock out of the fractalkine receptor leads to significantly smaller infarct volumes and less severe neurological deficits after middle cerebral artery occlusion (MCAO). In addition, fewer apoptotic neurons and reduced levels of reactive oxygen species (ROS) could be found. Microglial cells without the receptor displayed a significantly higher number of ramifications, indicating impaired development of activated microglial hypertrophic/amoeboid morphology [7, 8].

In vivo 2P-LSM and MRI

The development of in vivo ultrasensitive MRI and 2P-LSM has allowed the study of microglial functions under steady state in intact brains of living animals. In particular, in vivo imaging has revolutionized our understanding of neuroinflammation after damages to the brain [1–3]. By using long-term monitoring with in vivo 2P-LSM and ultrasensitive MRI, two non-invasive dynamic imaging modalities with resolution ranging from whole body to subcellular processes are available.

Inflammation is a hallmark of numerous neurological diseases exerting both deleterious and beneficial effects. Hence, non-invasive evaluation of inflammation is of particular interest for diagnosis and monitoring purposes. Following parenchymal injury, expressions of proinflammatory cytokines (especially by activated microglia) and adhesion molecules (including P/E-Selectins, Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) are upregulated, thus allowing recruitment of leukocytes in the brain parenchyma.

Previously, molecular magnetic resonance imaging (MRI) of inflammation using ultra-small particles of iron oxide (USPIO) has been described in both preclinical [9] and clinical studies [10]. Following intravenous injection, these USPIO are uptaken by circulating monocytes. Thus, subsequent accumulation of these cells can be revealed by iron-sensitive T2- or T2*-weighted MRI. The limits of this approach (time between injection and imaging, passive extravasation through altered blood-brain barrier and low sensitivity to detect early inflammation [9, 10] preclude its wide use in neurological disorders.

Following intravenous injection, these USPIO are phagocytozed by circulating monocytes. Thus, subsequent accumulation of these cells can be revealed by iron-sensitive T2- or T2*-weighted MRI. The limits of this approach (time between injection and imaging, passive extravasation through altered blood-brain barrier and low sensitivity to detect early inflammation [9, 10] preclude its wide use in neurological disorders. As an alternative, positron emission tomography (PET) using radioisotope-labeled probes has been successfully used to depict inflammation in several neurological disorders, including clinical studies. The most reported probe is [11C]-PK11195, targeting the translocator protein (TSPO), which is overexpressed by parenchymal activated microglia in inflammatory conditions. However, limited availability and low spatial resolution impairs the use of PET in routine clinical practice.

Among the proteins expressed by inflamed endothelial cells, VCAM-1 is a target of choice since it is not expressed by normal endothelium, and is upregulated in many neurological disorders, is localized at the luminal side of the endothelial cells and has been successfully imaged in previous studies by both MRI and ultrasound-based imaging. Additionally, VCAM-1 is the indirect target of Natalizumab, a monoclonal antibody used as a multiple sclerosis treatment.

Endothelial VCAM-1 and its ligand, α4β1 integrin, are key mediators of leukocyte recruitment. Recently the development and application of a novel molecular imaging probe that identifies VCAM-1 expression in mouse brain in vivo using MRI have been reported [11]. The specificity and potency of the contrast effects are dramatic and are derived from a combination of targeted delivery of a high payload of iron oxide to sites of early inflammation and rapid clearance of MPIO from the blood.

2P imaging and transgenic animals

2P-LSM

In vivo 2P-LSM, i.e. analyzing cellular responses in the living animal, is an exciting tool for neurological research as it can reveal how single cells within their local microenvironment respond to damage of the nervous system, highlighting the sequence of occurring events. This imaging approach provides significant advantages over conventional post-mortem studies of CNS trauma models, including the ability to directly trace the timing and behavior of highly dynamic cells, instead of deducing how cells change from ‘snapshots’ of fixed tissue. The decisive breakthrough for neuroimaging in live animals has come with the 2P-LSM technology [12] combined with sophisticated genetic tools to fluorescently label in vivo specific subsets of neural cells. 2P-imaging of the brain made the analysis of several neurodegenerative disease processes possible, including Alzheimer’s disease [13, 14], ALS [15] and artery occlusion in ischemic stroke [16, 17].

2P imaging of the functional neurovascular unit

Disruption of functional neurovascular coupling is a hallmark of ischemic stroke. The neurovascular unit
Imaging neuroinflammation after brain injuries by ultrasensitive MRI and two-photon laser-scanning microscopy

(NVU, a microenvironment) consists of neural cells (neurons, astrocytes, oligodendrocytes, microglia) that directly or indirectly interact with vascular cells (endothelial cells, pericytes and vascular smooth muscle cells). The maintenance of the stability of the BBB is one of the main functions of the NVU, established mainly through the interaction of pericytes and astrocytes [18, 19]. By combining transgenic animals with fluorescent protein expression and injection of dyes (Texas Red Dextran, variable size) to the tail vein, the vasculature and the adjacent cells building the neurovascular unit can be visualized (Figure 1).

![Figure 1 - The neurovascular unit. (A) This angiogram is a maximum intensity projection (MIP) of a 256-μm z-stack in the cortex of a wild type mouse. (B) MIP of a 18-μm z-stack recorded in the cortex of a TgN(GFAP-EGFP) mouse. (C–F) Snapshots of time-lapse recordings of microglia reaction to a laser-induced lesion (star in D). These images are MIPs of 40-μm z-stacks in the spinal cord of a TgH(CX3CR1-EGFP) mouse. In all images vessels were labeled by intravenous injection of Texas Red Dextran (70 000 kDa).](image)

Likewise, in vivo imaging of complex transgenic animals with multiple fluorescent protein expression in acute and chronic experiments has helped us to understand the interplay between astrocytes and oligodendrocytes following laser induced lesion or cortical stab wound. By using a double transgenic animal with green astrocytes and red oligodendrocytes [20, 21] it could be shown the effect of different acute injury models on glial cells. Astrocytes as well as oligodendrocytes are activated which can be visualized by an increase in the transgenic expression of the fluorescent proteins EGFP and PLP. By using double transgenic mice with neurons express EYFP and microglia EGFP, it could be shown that after small laser-evoked spinal cord injuries microglial cells respond in different time windows and accumulate around the lesion site in large aggregates (Figure 2).

![Figure 2 - Accumulation of microglial cell aggregates after spinal cord injury. (A–F) Laser-evoked spinal axon (red) transection activates adjacent microglia (green). Within minutes after the injury, first microglial processes move to the lesion site (arrow in B), but within less than 72 hours invading microglia form extended cell aggregates (F).](image)
2P-LSM and stroke

For investigation of cellular responses to ischemia-related insults, two different models are used: (1) the middle cerebral artery occlusion model (MCAO) or (2) ministrokes (laser-induced photothermolysis). Using the MCAO model perturbations of neuronal process morphology and local blood flow could be identified [22]. Inducing MCAO for a duration of 60 minutes produced a large core region with severe structural damage that did not recover after reperfusion as seen by 2P-LSM of transgenic mice with fluorescently labeled neurons. The core region is surrounded by a reversibly damage area (penumbra), which recovered after reperfusion and a structurally relative intact area with hypoperfusion (medial penumbra). Structural damage is seen as dendritic blebbing, which could be reverted in the penumbra region. Reperfusion of animals after 60 minutes of MCAO was not associated with exacerbation of damage [23]. In vivo imaging of neuronal dendrites also revealed a balanced and branch-specific remodeling of mature cortical pyramidal dendritic arbors after stroke [24].

Molecular imaging of inflammatory biomarkers after stroke by ultrasensitive MRI

Following parenchymal injury, expressions of proinflammatory cytokines (in particular the endothelial VCAM-1 and its ligand α4β1 integrin) control the recruitment of leukocytes into the brain parenchyma. Among the proteins expressed by inflamed endothelial cells, VCAM-1 is a target of choice since it is not expressed by normal endothelium, has been shown to be upregulated in many neurological disorders, is localized at the luminal side of the endothelial cells and has been successfully imaged in previous studies by both MRI and ultrasound-based imaging. Additionally, VCAM-1 is the indirect target of Natalizumab, a monoclonal antibody used as a multiple sclerosis treatment.

Endothelial vascular cell adhesion molecule-1 (VCAM-1) and its ligand, α4β1 integrin, are key mediators of leukocyte recruitment. Recently the development and application of a novel molecular imaging probe that identifies VCAM-1 expression in mouse brain in vivo using MRI have been reported [11]. The specificity and potency of the contrast effects are dramatic and are derived from a combination of targeted delivery of a high payload of iron oxide to sites of early inflammation and rapid clearance of MPIO from the blood (Figure 3).

Post-stroke neuroinflammation

Acute stroke causes a long-lasting, strong multiphasic inflammatory reaction, which involves the recruitment of local microglia. Activated microglia release chemotactants for infiltrating polymorphonuclear leukocytes (PMNs; neutrophilic granulocytes, neutrophils, monocytes/macrophages and T-cells) from the peripheral blood and exacerbate ischemic brain injury and play a role in the development of secondary injury after acute ischemic infarction [25]. The early phase of the inflammatory response (the first 10 days after injury) comprises infiltration of PMNs and T-cells and each cell population reaches its maximal abundance at specific time points. The second phase of cellular inflammation initiates two weeks after injury, peaks at two months, and remains detectable at six months post-injury [26].
Under pathological conditions, microglial functions are largely dependent on their activation stimuli; whereas short-lived mild microglia activation might help in tissue preservation, repair, and renewal, intense activation or chronic activation following an overt injury like spinal cord injury, optic nerve crush, or stroke is neurotoxic and may result in neuronal death and tissue loss [27–30]. More specifically, microglia-derived TNFα induces NPC apoptosis via a mitochondrial pathway regulated by the Bcl-2 family protein Bax. BH3-only proteins are known to play a key role in regulating Bax activation and we demonstrate that microglia-derived TNFα induces the expression of the BH3-only family member Puma in NPCs via an NF-kB-dependent mechanism [31].

**Neuroinflammation after stroke in aged subjects**

Normal aging is a situation characterized by a chronic low-grade, pro-inflammatory state [32], with an overexpression of systemic inflammatory factors, including proinflammatory cytokines [33, 34]. Age is the principal non-modifiable risk factor for stroke. The major factors involving the loss of regenerative capacity in the aged brain are an age-related decrease in neurogenesis, and the environmental hostility created by the inflammatory response to stroke. However, we and others could show that potential mechanisms for self-repair also operate albeit to a lesser extent in the post-ischemic aged brain. The process of cellular senescence can be an important additional contributor to chronic post-stroke injuries by creating a “primed” inflammatory environment in the brain [35–37]. Recent work suggests that perfusion deficits in the elderly can trigger microglial activation and subsequent neuroinflammation [38, 39] ultimately resulting in demyelination and neurodegeneration [40].

**Post-stroke anti-inflammatory therapies**

Despite the approved indication of systemic thrombolytic therapy for the acute management of ischemic stroke, its use is limited given a strict eligibility criteria and a risk for hemorrhagic transformation as a feared adverse effect. Systemic thrombolytic therapy has been shown to amplify microglia recruitment early after stroke in association with a rapid CCL3 production. This early response may explain the higher mortality after rtPA treatment compared with saline treatment during the first day of reperfusion [41].

Persistent neuronal death causes a prolonged neuro-inflammatory response in the infarcted area. We have to find ways to minimize functional impairment by preventing neuronal death, which occurs massively in the first three days after stroke and continues for days to weeks following the acute event [42, 43].

**Anti-inflammatory drug therapies**

Earlier studies have suggested that neuroinflammation alone inhibits neurogenesis and that inflammatory blockade with Indomethacin, a common non-steroidal anti-inflammatory drug, restores neurogenesis after endotoxin-induced inflammation and augments neurogenesis after cranial irradiation [42, 43]. Recent studies have reported that targeting the inflammatory response to ischemic injury limits the expansion of the lesion and increases the survival of neurons after stroke [44–46]. For example, Ibuprofen was found to down-regulate the traumatic brain injury (TBI)-induced inflammatory response. In addition, migrating neuroblasts from transplanted cells were observed near the contusion and in the ipsilateral hippocampus in Ibuprofen-treated animals only, suggesting that the anti-inflammatory treatment had beneficial effects on graft survival and/or differentiation [47].

Minocycline is a tetracycline anti-infective agent that shows promise for its neuroprotective effects in multiple animal models and three human trials. Minocycline at a dosage of 200 mg was administered orally for five days. The therapeutic window of time was six to 24 hours after onset of stroke [48]. Minocycline alone or in combination with tissue plasminogen activator also has other beneficial effects including a reduction in apoptosis, neuroinflammation, infarct size, and vascular injury [49]. Its anti-inflammatory effects are mediated through inhibition of inducible form of nitric oxide synthase and p38 mitogen-activated protein kinase, reduction of glutamate toxicity, and inhibition of microglial activation [50]. Quite recently, pre- or post-stroke treatment with the PI3Kδ inhibitor CAL-101 of kinase-dead PI3Kδ [p110δ/D910A/D910A] or wild-type mice led to improved functional outcome along with reduced infarct size, decreased leukocyte infiltration, and reduced TNF levels [51]. Likewise, housing rodents in an enriched environment provides multi-sensory stimulation to the brain and enhances functional recovery after experimental stroke, also depressing the release of cytokines and chemokines in the peri-infarct [52].

**Hypothermia in animal models and stroke patients**

A viable alternative to conventional drug-based therapy of neuroinflammation is physical cooling (hypothermia), either confined to the head or including the entire body [53]. Cooling decreases brain oxygen consumption, glucose metabolism, lactate production and prevents the development of acidosis [54]. Mild to moderate hypothermia has been experimentally shown to reduce neuronal death after experimental ischemia and improve motor function [55]. Hydrogen sulfide does not smell well and for this reason, many groups are using H2S donors such as 5-(4-methoxyphenyl)-3H-1,2-dithiole-3-thione (ADT) and sodium hydrosulfide (NaHS). Using this approach it was recently shown that H2S donors protected BBB integrity following experimental stroke possibly by acting through NF-κB inhibition to suppress neuroinflammation induction of MMP9 and NOX4-derived free radicals [56].

**Stem cell therapy of stroke**

In addition, cell therapy itself can be used during the first week post-stroke to limit neuroinflammation in animal models [57–59]. A recent study emphasized the crucial importance of timing and cell dose for successful post-stroke treatment using BM MSC. The study found that transplantation of BMSCs at three and 24 hours, but not seven days after focal ischemia, significantly reduced the lesion volume and improved motor deficits. Similarly, transplanted cells in the range 1×10⁶ to 10⁷, but not at
1×10^4 to 10^5, significantly improved functional outcome after stroke. In addition to inhibiting macrophages/microglia activation in the ischemic brain, BMSC transplantation profoundly reduced infiltration of gamma delta T (γδT) cells, which are detrimental to the ischemic brain, and significantly increased regulatory T-cells (Tregs), along with altered Treg-associated cytokines in the ischemic brain [56]. Furthermore, the survival of MSCs was found to be very low (between 0 and 30%) and was highly dependent on the injection route/site (intravenous, intracerebral or intrathecal) and cell source (auto-, allo- or xenogeneic) [60]. Immune-related factors that impair neuronal survival and induce neuronal death also inhibit regeneration; therefore, immunomodulation should benefit stem cell therapy. Both in vitro and after transplantation in vivo, NSPCs not only form neural cells for replacement but also exert immunomodulatory and trophic effects, so-called “therapeutic plasticity” [46, 61].

Attractive therapeutic strategies stimulating and finally enhancing the natural post-stroke regeneration process include methods of training such as physio- or rehabilitative therapy as well as methods of cellular therapy [62–64]. Mesenchymal stromal cells (MSCs), derived either from bone marrow or from adipose tissue, have been shown to ameliorate the clinical outcome in experimental model of cerebral ischemia [64, 65]. Administration of MSCs in acute stroke animal models markedly decreased brain infarct size, improved neurological function by enhancing neurogenesis, and showed anti-inflammatory and antiapoptotic effects. Additionally, initial clinical studies using intravenously delivered MSCs have been initiated in human subjects with stroke [66].

The mechanisms by which MSCs may ameliorate infarcted brain tissue seem related more to the capacity of MSCs to release neuroprotective factors (paracrine mechanism) than to their capacity to replace damaged neural cells through their transdifferentiation properties. It has been shown that MSCs are able to release several angiogenic and neurotrophic factors, as well as anti-inflammatory molecules [67]. At this regard, it has been shown that when stimulated by inflammatory cytokines, MSCs increased their anti-inflammatory capacity, suggesting that MSCs may even improve their efficacy when localized in an inflammatory microenvironment in vivo [68]. Additionally, MSCs seem to have a good capacity to home to ischemic brain when administered through a systemic route [69].

Mild neuroinflammation can be beneficial for regenerative events aimed at functional restoration after stroke [70]. However, persistent post-stroke neuroinflammation results in decreased proliferation of the newly born NSPCs and ineffective integration into the circuitry of the re-organized brain area [30]. Moreover, a number of studies have demonstrated that neuroinflammatory processes can induce apoptosis in NPCCs and immature neurons [42, 71] 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 [43, 72, 73]. More recently studies directly implicated TNFα produced by lipopolysaccharide-activated microglia is as a key determinant in microglia induced-apoptosis in mouse NPCCs in vitro and in vivo [31].

Since stroke afflicts mostly the elderly, it is highly desirable to test the efficacy of cell therapy in the microenvironment of aged brains that is generally refractory to regeneration. In particular, stem cells from the bone marrow allow an autologous transplantation approach that can be translated in the near future to the clinical situation. Such a bone marrow-derived therapy includes the grafting of stem cells as well as the delayed induction of endogenous stem cell mobilization and homing by the stem cell mobilizer Granulocyte-colony Stimulating Factor (G-CSF). We tested the hypothesis that grafting of bone marrow-derived pre-differentiated mesenchymal cells (BM MSC) in G-CSF-treated animals improves the long-term functional outcome in aged rodents. To this end, G-CSF alone (50 μg/kg) or in combination with a single dose (106 cells) of rat BM MSC were administered intravenously to Sprague–Dawley rats at six hour post-stroke. Infarct volume was measured by MRI at three and 48 days post-stroke and additionally by immunohistochemistry at day 56 (Figure 4).

Functional recovery was tested during the entire post-stroke survival period of 56 days. Daily treatment for post-stroke aged rats with G-CSF led to a robust and consistent improvement of neurological function after 28 days. The combination therapy also led to robust angiogenesis in the formerly infarct core and beyond in the “islet of regeneration”. However, the G-CSF + BM MSC combination may not impact at all on the spatial reference-memory task or infarct volume and therefore did not further improve the post-stroke recovery.

Conclusions

The efficacy of all cell therapies so far is discouragingly low mainly because the time course of interactions between host neuroinflammation, which is considered to be a major obstacle to exogenous-mediated neuronal pre-cursor cells, and stroke-induced neurogenesis or exogenously administered stem cells, is virtually unknown. These findings strongly suggest that anti-neuroinflammatory therapy is a potential target to promote regeneration and repair in diverse injury and neurodegenerative conditions by stem cell therapy. The challenge now is to determine in more detail the cross-talk between different populations of immune cells and endogenous and grafted NSPCs at different phases after stroke. Further, we hypothesize that in a real clinical situation involving older post-stroke patients, successful regenerative therapies would have to be carried out for a much longer time, perhaps between 6–12 months.

References


Imaging neuroinflammation after brain injuries by ultrasensitive MRI and two-photon laser-scanning microscopy

Figure 4 – (Upper panel). Edema and stroke volumes by MRI and NeuN immunohistochetometry. (a–c) Perilesional brain edema at day 3 post-stroke, as defined by the region of T2 hyperintensity (A) was not significantly reduced by any treatment. (d–f) The second MRI done at day 48 (B) post-stroke revealed much smaller infarcts. (Lower panel). Migration routes and phenotyping. In the ipsilateral hemisphere, the injected human BMSCs migrated toward the peri-infarcted area via the corpus callosum as shown for CD166-positive cells (A, arrows) and CD105-positive cells (F, arrows). In our model, the cell entered the injured brain via the lateral ventricle as shown by the CD166-positive cells (B). A fraction (about 1%) of the injected CD166- and CD105-positive cells reached the infarcted area (C and E, arrows) where they were intermingled with surviving or degenerating neuronal nuclei (C, arrowheads). Noteworthy was also the presence of immunopositivity for human nuclei (D, arrows) that were dispersed between the rat nuclei in the infarcted area (D, arrowheads).


Effects of minocycline plus tissue plasminogen activator combination therapy after focal embolic stroke in type 1 diabetic rats, Stroke, 2013, 44(3):745–752.


Imaging neuroinflammation after brain injuries by ultrasensitive MRI and two-photon laser-scanning microscopy


Corresponding author
Aurel Popa-Wagner, MD, PhD, Department of Psychiatry, University of Medicine Rostock, Gehlsheimerstr. 20, 18147 Rostock, Germany; Fax +49–381–494–9507, e-mail: aurel.popa-wagnerap@med.uni-rostock.de

Received: May 20, 2014
Accepted: September 23, 2014