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Oral presentations

Translating mechanistic insight into therapy

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The last two decades has witnessed spectacular advances in our understanding of the molecular mechanisms which are involved in ischaemic cell death and of pharmacological approaches which could markedly modify outcome in experimental models. In contrast, translating this mechanistic insight into effective therapy for stroke in man has been disappointing. This lecture will highlight some of the opportunities which are available to meet the challenges of translating basic neuroscience progress into new therapies.

The economic environment and pharmaceutical industry has changed fundamentally since the first neuroprotective drugs were administered to humans. Government agencies around the world now require more potential economic benefit from research rather than the advancement of knowledge. Stroke is no longer a high priority for big pharmas. We must recognise the fundamental alterations in the research environment and utilise our available assets to reengage pharmaceutical industry with our research areas.

The quality of our research needs to be enhanced and the leadership provided by the Journal of Cerebral Blood Flow and Metabolism in promoting good laboratory practices needs to be widely recognised.

Middle cerebral artery occlusion models (along with global ischaemic models) have been particularly valuable to define molecular mechanisms pertinent to human stroke. There are additional therapeutic targets for ischaemia research (e.g. age related cognitive decline) which require new models (e.g. carotid stenosis models in mice) and a shift of research emphasis (from rapidly evolving changes in neuronal perikarya to slowly evolving pathology in myelinated axons).

Ischaemic research has been slow to adopt new technologies; for example shotgun proteomics offers great potential in exploring the overall cellular response to ischaemic challenges. Pharmacological modulation of protein-protein interactions with low molecular weight drugs is feasible as our recent studies with baxnucleophosmin illustrates.The challenge of translating mechanistic insight into new therapeutic strategies can be met but it will require sustained action on our part. On all of the issues raised in the lecture.

A new era for MSC therapy

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Mesenchymal stromal cells (MSCs) are spindle-shaped, plastic adherent, multipotent cells that can be isolated from bone marrow, cord blood, adipose, placenta, and many other tissues. Based on their remarkable self-renewal capacity and broad differentiation potential *in vitro*, these cells were considered to be stem cells and MSC research was focused on rebuilding diseased or damaged tissue with autologous or allogeneic MSCs. We now understand that the biologic effects of systemically infused MSCs seem to be mediated through the release of soluble molecules that stimulate reparative activity by host cells in the host tissue. This new paradigm of MSC therapeutic activity shifts our focus to investigating what soluble mediators are produced by ex vivo expanded MSCs. Moreover, the tissue source from where the MSCs are isolated, in addition to the culture expansion conditions, may significantly impact the MSC secretome. Thus, there is a great need to reinvestigate MSC isolation and expansion. In the central nervous system, MSCs have already been shown to stimulate outgrowth of host neurons after local implantation in mice. Furthermore, MSCs stimulate the endogenous secretion of NT-4/5, NGF, VEGF, CNTF, FGF-2, and may secrete BDNF in response to appropriate environmental cues. Finally, MSCs have been reported to benefit experimental induced several CNS disorders in animal models, most notably stroke. We are embarking into a new era of MSC research that may reveal far greater clinical advances that we have realized over the past two decades. The clinical poten-

tial is just beginning to be understood and the nervous system will likely become a one of the most benefited by MSC therapy.

Factors such as regulatory proteins, mRNA and miRNA are transported by stem cell specific microparticles that are released into the cerebrospinal fluid following traumatic brain injury (TBI)

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Intercellular exchange of protein and RNA-containing microparticles is an increasingly important mode of cell-cell communication. Microparticles, which include exosomes, micro-vesicles, apoptotic bodies and apoptotic microparticles, are small (50–400 nm in diameter), membranous vesicles that can contain DNA, RNA, miRNA, intracellular proteins and express extracellular surface markers from the parental cells.

Our primary aim is to elucidate the role of microparticles in damage induced neurogenesis following traumatic brain injury in vivo.

Material and Methods: Cerebrospinal fluid was serially centrifuged. Pelleted microparticles were analysed by FACS, electron microscopy, RT-PCR and mass spectroscopy. Quantification of miRNA was performed by Small RNA Chip (Agilent Tech.). Specific miRNAs were identified by miRNA microarrays (Affymetrix).

Results: We verified the presence of cerebral cell derived microparticles in cerebrospinal fluid of healthy volunteers and patients of TBI by FACS analysis and electron microscopy. Microparticles contained RNA, miRNA and protein. RNA was not susceptible to RNase digestion underlining RNA to be contained in protective vesicles. Approximately 50% of the RNA content was demonstrated to be pre- and miRNA. A variety of specific miRNA species indicated in the regulation of regenerative processes were identified. Furthermore, RT-PCR analysis revealed cerebral microparticles to carry transcripts for MAP2, ß-actin and CaMKII. MAP2 mRNA was not detected in cerebral microparticles of healthy controls. The presence of ß-actin and CaMKII in microparticles might indicate neuronal RNA granules to be also packed into microparticles. Proteomic analysis indicated microparticles to carry proteins involved in motility (ankyrin-3), membrane regulation (stabilin-2, synaptotagmin), neuronal development (growth factor independence-1), as well as typical neuronal receptors (glutamate receptor). Within the first three days following traumatic brain injury $14\% \pm 3\%$ of microparticles in cerebrospinal fluid was CD133+, indicating a substantial fraction of microparticles to be derived from neuronal precursor cells.

Conclusion: We were able to demonstrate for the first time the release of stem cell derived microparticles into cerebrospinal fluid. The detection of specific RNA transcripts and pre/miRNA underlines their predicted role for microparticles in cell-cell communication. To our knowledge there are no reports to date on the role of microparticles in the cerebral environment. We are presently studying the role of microparticle in endogenous neurogenesis following cerebral injury.

Adipokines in post-stroke patients with hip fracture: potential therapeutic targets?

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Background: Stroke is a major risk factor for hip fracture (HP). Although in the last decade numerous studies linked adipokines, especially leptin and adiponectin, with both central nervous system functions and bone homeostasis, the complex interactions between adipokines, stroke and bones remain poorly understood. The significance of adipokines in post-stroke HF is unknown.

Objectives: To examine the relationship between serum leptin, adiponectin and resistin concentrations and markers of bone metabolism in HF patients and to delineate any differences between patients with and without history of stroke.

Methods: In 294 consecutive patients with HF (mean age 81.9 7.7 years; 71% women) serum levels of leptin, adiponectin and resistin (ELISA methods), markers of bone formation (osteocalcin, bone specific alkaline phosphatase), mineral metabolism, PTH, 25(OH)vitamin D and urine markers of bone resorption (deoxypyridinoline and cross-linked N-telopeptide of type I collagen, both normalised to urinary creatinine, DPD/Cr, NTx/Cr, respectively) were measured.

Results: Post-stoke HF patients ($n = 39$, 13.3%) compared to the rest of the cohort have markedly higher serum PTH levels ($+15.8\%$; $p = 0.040$) and accel-

erated bone remodelling (+45.3%; $p = 0.009$) mainly due to increased resorption (+33.3%; $p = 0.004$). In multivariate analysis after adjustment for age, sex, HF type, comorbidities and laboratory parameters, including PTH and 25(OH) vitamin D, history of stroke was significantly associated with higher serum resistin level ($>$ 16.26 ng/ml, median level; OR = 3.81; 95% CI 1.50–9.64; $p = 0.005$). No significant associations were observed for leptin and adiponectin levels and history of stroke. Resistin (log-transformed) was an independent (inverse) predictor of serum osteocalcin ($= -3.399$; $p = 0.018$). Adiponectin positively correlated (Pearson's coefficient) with PTH in both groups with ($r = 0.394$; $p = 0.019$) and without ($r = 0.193$; $p = 0.006$) history of stroke. Only in post-stroke HF patients leptin negatively correlated with both urine resorption markers DPD/Cr ($r = -0.466$; $p = 0.007$) and NTx/Cr ($r = -0.403$; $p = 0.022$), and serum phosphate ($r = -0.371$; $p = 0.026$). In HF patients without a history of stroke osteocalcin was positively associated with leptin ($r = 0.141$; $p = 0.031$), leptin/adiponectin ratio (0.155; $p = 0.025$) and leptin/resistin ratio ($r = 0.180$; $p = 0.001$).

Conclusions: In older HF patients higher resistin circulating levels are strongly associated with history of stroke and predictive (negatively) of serum osteocalcin levels. In post-stroke HF patients bone resorption indices are higher than in the rest of the cohort, and serum leptin concentrations are inversely associated with resorption markers. Coupled with data on the role of adipokine dysregulation in stroke, our findings suggest that pharmacological manipulation of adipokines (inhibition of resistin actions and replacement of leptin) may be a novel approach to prevent and treat post-stroke HF.

Thus bad begins and worse remains behind': Systemic effects of stroke as target for brain protection

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Stroke is a major cause of morbidity and mortality worldwide. With a very strong focus on postischemic signaling in the brain tremendous progress has been made in the last decades in our basic understanding of the pathobiology of cerebral ischemia. However, for many reasons and despite tremendous efforts, protecting the brain after stroke by targeting cascades of damage has remained an unmet challenge. Only recently it has become clear that other organ systems, more easily accessible to therapeutic approaches than the brain, also have an important impact on outcome after stroke. Stroke affects the normally well balanced interplay of two supersystems – the nervous and the immune system. Ongoing research elucidated some of the signals and mechanisms involved, and was able to demonstrate that brain-immune interactions are highly relevant for functional outcome after stroke. The cardiovascular system impacts on post-stroke recovery, not only because of the potential of recurring events, but also because it is involved in revascularizing ischemic areas and potentially also in regeneration and repair (e.g. via EPCs). Stroke also affects systemic metabolism, with potentially important consequences on body composition and short as well as long term outcome. I will review our current knowledge on the impact of systemic alterations after stroke. I will focus on the immune system and infection, the cardiovascular system, as well as systemic metabolism, and speculate about potential novel avenues of therapy which might result from our growing insight into these interactions.

Neuroprotective cytokines in cerebral ischemia

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The pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin-1 (IL-1) are both well known for their involvement in the pathophysiology of stroke. While IL-1 potentiates ischemia-induced neurotoxicity, an effect that can be antagonised by the IL-1 receptor antagonist (IL-1Ra), which is itself a cytokine, there is still controversy about the impact of TNF on ischemic neurons. To better understand the mechanism of action of these cytokines, we have investigated the cellular production of IL-1/IL-1Ra and TNF, and the receptors of these cytokines. We among other report that IL-1b and TNF are produced in segregated populations of microglia-macrophages, whereas TNF and IL-1Ra are to a large extent co-expressed in microglia. Further, in line with recent reports from our group of a neuroprotective effect of microglial-derived, but not macrophage-derived, TNF, we now show evidence of a neuroprotective effect of microglial-derived IL-1Ra. Unlike TNF, which is produced by both microglia and blood-derived macrophages, IL-1Ra is predominantly produced by microglia, and in significantly lower levels by blood-borne leukocytes. This raises the possibility

to increase the level of IL-1Ra in the borderzone and within the ischemic infarct by introduction of IL-1Ra producing cells. In this talk, we will report new observations from our own group on the production and role of IL-1/IL-1Ra and TNF in cerebral ischemia in the context of current knowledge on these cytokines in stroke.

Recruitment of monocytes are needed for neuroprotection and repair

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For decades, the central nervous system (CNS) was viewed as an autonomous unit, nourished by the blood, and shielded from pathogens and toxins present in the circulation. In addition, since it is equipped with its own innate immune cell population, the microglia, which was believed to be capable of fully providing the brain's needs for defense and protection, any further infiltration of blood macrophages was viewed as a sign of pathology that should be mitigated. Based on a decade of experimental evidence showing that the peripheral immune cells (both CD4+ T cells that recognize brain antigens, and blood-monocytes) are needed for brain plasticity in health and disease, and through an understanding of how and where this immune support occurs, our group has proposed that the central nervous system (CNS) is critically dependent on these peripheral immune cells for neuronal survival and repair, for neurogenesis and oligodendrogenesis from resident neural stem cells, for coping with a stressful environment, and for fighting off acute and chronic neurodegenerative conditions. Insufficient immunity or its malfunction can impact cognitive performance, resilience to stress, emergence of developmental neuropsychological disorders (e.g., Schizophrenia), and the onset and progression of neurodegenerative diseases such as Alzheimer's disease, glaucoma, age-related dementia, and ALS. Boosting of peripheral immunity under pathological conditions including acute CNS trauma, Alzheimer's disease, depression, and glaucoma was found to be an effective means of enhancing recruitment of monocytes that locally displayed a distinctive role than activated resident microglia. These monocytes locally displayed an anti-inflammatory phenotype (also known as M-2 or 'alternatively-activated macrophages'). Such macrophages in turn terminated the microglia response and contributed to a scar resolution. In Alzheimer's disease, vaccination with weak antigens resulted in recruitment of monocytes that modified the local milieu in terms of cytokine profile and growth factor composition. Taken together, our results suggest that blood monocytes are key players of neuroprotection and neurorepair; their effect reflects timing, context and location. The limited recruitment will be discussed

Imaging the innate immune cells after experimental stroke

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Cerebral ischemia is accompanied by an acute inflammation, involving the activation of microglia and the infiltration of neutrophil granulocytes and monocytes into the brain. How these different immune cell types contribute to the neuronal outcome after cerebral ischemia is still under discussion. Various significant actions of immune cells are just to reveal by imaging them either *in vitro/ex vivo* or *in vivo*. We developed a postischemic *ex vivo* model of immune cell (fluorescently labeled) application on hippocampal slices with eYFP expression in neurons. We observed two significant mechanisms how microglia protect neurons after ischemia. On the one hand microglia were found ischemia induced in close proximity or in physical cell-cell contact to the neurons and on the other hand microglia eliminated infiltrating neutrophil granulocytes very fast and efficient. Blocking both properties yielded in an exacerbation of neuronal damage. To test our hypothesis *in vivo* we generated a mouse transgenic for neutrophils (Lys-EGFP) and microglia (CX3CR1-EGFP). For experimental cerebral ischemia we used a model of permanent middle cerebral artery occlusion combined with an occlusion of the common carotid arteries for 20 min. With intracranial two-photon microscopy (TPM) we are able to image these cells to a depth of 300 μm *in vivo* after ischemic lesions. To date we observed a rapid infiltration of neutrophils and a very fast response of microglia to damaged vessels after ischemia. In more detail neutrophil granulocytes adhere promptly to the vessels and subsequently invade the cerebral parenchyma. Furthermore microglia seemed to shield inflamed vessels by sending their processes. This approach is suitable to answer a wide range of questions how immune cells respond to cerebral ischemic events and might contribute to gain intelligence for developing suitable therapeutic strategies.

Innate immunity and inflammation in stroke

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Objectives: Stroke induces inflammatory reactions associated with release of cytokines, induction of adhesion molecules, and activation of matrix proteases. These molecular changes cause activation of resident glia, alterations blood-brain barrier permeability, and infiltration of leukocytes, which, overall, are thought to contribute to damage progression. Acute brain damage can trigger innate immune responses in the absence of pathogens by generating 'danger signals'. Neuronal necrosis causes the appearance in the extracellular environment of normally intracellular molecules, which can activate pattern recognition receptors, such as toll-like receptors (TLR), in surrounding cells. Also, disruption of the extracellular matrix after protease-mediated cleavage of matrix proteins might unveil epitopes that could activate innate immune receptors. Several lines of evidence supports that components of the innate immune system participate in brain damage after stroke. We have been interesting in studying whether innate immune responses mediated by TLR and the complement system participate in the early response to stroke, whether they mediate proinflammatory responses, and whether they are involved in brain damage.

Material and methods: We use experimental models of brain ischemia in mice and rats. We induce inflammation by activating TLR4 with the bacterial lipopolysaccharide (LPS) in the brain of rodents or by treating cultured glial cells. We use animals deficient in molecules of the TLR4 signalling pathway, such as MyD88 or Stat1, to study signal transduction, and we use animals deficient in mannose-binding lectin (MBL), which is a main component of the lectin pathway for complement activation. We also studied genetic polymorphisms related to innate immune responses in stroke patients.

Results: Signalling though TLRs triggers a complex network of molecular responses and the rapid release of proinflammatory mediators that can exacerbate brain damage after ischemia. Furthermore, the complement system also contributes to brain damage after stroke, possibly though complement activation in the blood and on the surface of injured cells via different activation pathways, including the lectin pathway. Our findings show that genetic deficiency in mannose binding lectin (MBL) is beneficial in stroke in animals and in humans.

Conclusions: Our findings support that acute brain damage alerts the innate immune system and this leads to the massive release of proinflammatory mediators in the absence of invading pathogens. However, in view of recent findings evidencing that the innate immune system might play a role in regenerative processes, we believe that treatments directed to inhibit innate immune responses after stroke should have a certain temporal window of intervention, since while they might be beneficial in the acute phases they might impair regenerative process in the long term.

Molecular dissection of reactive astrogliosis and glial scar formation

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Although reactive astrogliosis is a ubiquitous feature of the CNS response to all forms of injury and disease, until recently this response has been poorly defined and its mechanisms poorly understood. Genetic tools are now enabling the molecular dissection of the functions and mechanisms of reactive astrogliosis *in vivo*. This presentation will examine findings from our and other laboratories indicating that reactive astrogliosis is a complex, multifaceted process comprising numerous potential cellular and molecular changes with a wide range of potential effects regulated by different signaling molecules. It is now clear that reactive astrogliosis is not a simple all-or-none phenomenon but is a finely gradated continuum of changes that occur in context dependent manners regulated by specific signaling events. These changes range from reversible alterations in gene expression and cell hypertrophy with preservation of cellular domains and tissue structure, to long lasting scar formation with rearrangement of tissue structure. Although reactive astrocytes have often been regarded as uniformly detrimental to clinical outcome, it is now clear that reactive astrogliosis, including scar formation, can exert both beneficial and detrimental effects in a context-dependent manner determined by specific molecular signaling cascades. In addition, increasing evidence points towards the potential that reactive astrogliosis may play either primary or contributing roles in a wide variety of CNS disorders via loss of normal astrocyte functions or gain of abnormal effects. A better understanding of astrocyte signaling mechanisms and the mechanisms of reactive astrogliosis has the po-

tential to open the door to identifying many molecules that might serve as novel therapeutic targets for a wide range of neurological disorders.

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Modulation of glial signal transduction as therapeutic strategy for neuroprotection

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The common pathophysiological hallmarks of neurodegenerative disorders are activation of microglia and astrogliosis, infiltration of immune cells and activation of the adaptive immune system. These processes progress by expression of cytokines, adhesion molecules, proteases and other inflammation mediators. In response to brain injury or infection, intracellular signaling pathways are activated in microglia, which turn on inflammatory and antigen presenting cell functions. Different extrinsic signals may shape microglial activation towards different phenotypes under pathological conditions. Mechanisms responsible and controlling a shift from beneficial to detrimental microglia phenotype are poorly known. Identification of signaling pathways and transcription regulators which may serve as "master switches", contributing to discrete phenotypes will allow to target specific functions of microglia. Using primary rat microglial cultures we characterized activities of major signaling pathways (AKT, FAK, MAPK kinases), transcription regulators (NF-κB, STAT) and global gene expression driven to inflammatory (induced by lipopolysaccharide – LPS) or cytoprotective phenotype. Computational analyses revealed different patterns of expression of genes encoding cytokines/chemokines and transcription regulators suggesting distinctive genomic responses. This was confirmed by determination of profiles of pro- or anti-inflammatory cytokines in brain extracts from mice subjected to different insults and gene expression in magnetically sorted CD11b positive cells from brains. We postulate that distinct actions of microglia may be due to activation/differentiation to distinct phenotypes: pro-inflammatory M1 or cell protective, immunosuppressive M2 phenotype associated with differential expression of genes and production of specific proteins. Using primary glial cultures we identified two soluble proteins acting via integrin receptors which enhance cytoprotective phenotype of microglia, stimulate its proliferation, migration and phagocytosis in primary microglial cultures. Those proteins were able to interfere with inflammatory signaling induced in microglial cells by LPS or interferon gamma. Furthermore, those proteins modulated signal transduction underlying inflammatory cytokine stimulated astrogliosis *in vitro*. Developing functionally manipulated microglial cells can be employed to convey neuroprotection/neurorepair. Understanding of signal transduction involved in glia-mediated inflammation allows a use of compounds which can target specifically those pathways. Identification of a crucial role of MAPK signaling pathways in activation of proinflammatory phenotype of glial cells allows targeting with a battery of inhibitors. Small molecule inhibitors of specific MAPK pathways, capable of reducing both the synthesis of inflammation mediators and inflammatory cytokine signaling, are potent modulators of brain inflammation and gliosis in neurological disorders.

In vivo imaging of cerebral potassium metabolism in focal cerebral ischemia in rats using 201TlDDC-SPECT

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Focal cerebral ischemia is accompanied by severe alterations in potassium (K^+)-metabolism in the affected region. Monitoring these alterations *in vivo* could be of substantial interest in preclinical research as well as for diagnostic purposes in clinical applications.

In principle, *in vivo* monitoring of K^+-metabolism is possible using suitable isotopes of the K^+-analogues rubidium or thallium. Due to the poor blood-brain barrier permeability of K^+ and K^+-analogues, however, little use has been made thus far of these tracers for imaging cerebral potassium metabolism.

We here present a novel approach for *in vivo* imaging of cerebral K^+-metabolism in focal cerebral ischemia. We intravenously injected rats with the lipophilic chelate complex 201-thallium diethyldithiocarbamate (201TlDDC) after induction of focal cerebral ischemia

by endothelin-mediated middle cerebral artery occlusion (MCAO). We monitored the 201Tl-distribution in the rat brains at various time points after 201TlDDC-injection using a small-animal SPECT/CT scanner.

We had previously shown using histochemical techniques that, after crossing the blood-brain barrier, Tl^+ is released from TlDDC and that neurons take up the ion in an activity-dependent manner. We reasoned that, conversely, in cerebral ischemia due to breakdown of Na,K-ATPase activity and K^+-gradients Tl^+-uptake will be reduced in the infarcted area and that, with infarct progression, Tl^+ will be lost from the tissue. We here show using SPECT-imaging that upon MCAO there is in fact a reduced 201Tl-uptake *in vivo* on the lesioned side as well as a continuous loss of 201Tl from the lesioned area during the first hours after onset of ischemia. The definite lesion is characterized by a marked reduction in 201Tl-content.

Our findings suggest that 201TlDDC is a useful tracer for SPECT-imaging of tissue viability, lesion growth and lesion size in focal cerebral ischemia. Using high-resolution multi-pinhole SPECT-imaging the patterns of 201Tl redistributions in rodent brains can be imaged with millimeter or submillimeter spatial resolutions. Small-animal 201TlDDC-SPECT can provide novel insights into the spatiotemporal dynamics of K^+-metabolism in focal cerebral ischemia.

Efficient transfection of a network of differentiated primary mammalian neurons by Nucleofection®

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Efficient delivery of biomolecules, such as DNA or RNAi substrates, into neural cells is a promising tool to study a broad spectrum of cellular functions. Primary mammalian neural cells can be cultured *in vitro* and exhibit pivotal functions when grown in adherence. For the transfection of adherent neurons a variety of methods exist which do not achieve high transfection efficiencies. Gene transfer by viral methods can be efficient for neural cells but is laborious, time-consuming and cost-intensive. The non-viral electroporation-based Amaxa® Nucleofector® Technology allows for efficient transfection of primary neural cells directly after isolation, combined with high viability and functionality.

Here we present a technology innovation which allows for efficient adherent Nucleofection® of primary mammalian neurons attached to their substratum. This has been achieved by the development of an enhanced version of the Nucleocuvette® Modules for use with the 96-well Shuttle® System. The wells of these modules are designed for cultivation and Nucleofection® and analysis of adherent neural cells *in situ*. This allows continuous cultivation of the neuronal cells for up to several weeks by eliminating the need for enzymatic or mechanical removal of the cells from their growth substrate. Furthermore, the cells can be transfected and analyzed at various time points during culture and thus maturation stages *in vitro*. The modified modules allow the analysis of the cells by light and fluorescence microscopy or by plate readers for absorption, luminescence or fluorescence assays. Analysis can be performed at any time point after transfection.

With up to 50% transfection efficiency and high viability for various primary neurons, e.g. embryonic rat cortical and hippocampal neurons and mouse cortical neurons, adherent transfection using the new Nucleocuvette® AD modules provides a new tool for studying primary mammalian neurons without interruption of their physiological state.

Mitochondrial Na^+-dependent Ca^{2+} efflux as a target of estradiol neuroprotective action

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Objectives: Several neural cells specific activities such as rapid channel functions, neurotransmitter release and synaptic plasticity as well as various physiological processes during cell metabolism, differentiation and cell death depend on intracellular Ca^{2+} concentration. Due to specific transport mechanisms and large Ca^{2+} storage capacity, mitochondria are organelles critical for Ca^{2+} buffering in neuronal cells. The goals of this study were to examine the rapid non-genomic effect of 17β-estradiol (E2) on Ca^{2+} sequestration in synaptosomal mitochondria isolated from brain steam of ovariectomised rats and to determine if, and to what extent, E2 receptors participated in mitochondrial Ca^{2+} transport modulation by E2 *in vitro*.

Materials and methods: Radioactive labeled calcium ($^{45}Ca^{2+}$) was used for Ca^{2+} movements monitoring. After pre-incubation at 22°C for 10 min in medium containing: 300 mmol/l mannitol, 10 mmol/l KCl, 1 mmol/l maleate, 5 mmol/l glutamate and 10 mmol/l Tris-HCl, pH 7.4, Ca^{2+} influx into synaptosomal mitochondria (0.2 mg protein/ml) started after 0.2 mol/l $CaCl_2$ (24.3–27.6 kBq $^{45}CaCl_2$) addition and the reaction lasted for 5 min. Ca^{2+} efflux from Ca^{2+}-preloaded mitochondria was initiated by 20 mmol/l NaCl and 0.2 mmol/l EDTA. The Ca^{2+} retention in mitochondria was calculated from radioactivity counting. The E2 effects on Ca^{2+} efflux were tracked, when mitochondria were incubated with 0.5 nmol/l E2 for 10 min, before Na^+/EDTA efflux initiation. The effects of ER antagonist 7α,17β-[9[(4,4,5,5,5-pentafluoropentyl) sulfinyl] nonyl] estra-1,3,5(10)-triene 3,17-diol -ICI 182,780 (1 μmol/l), ERα agonist 4,4‘,4“-(4-propyl-[1H]-pyrazole-1,3,5-triyl) *tris*phenol -PPT (10 nmol/l) and ERβ agonist 2,3-*bis*(4-hydroxyphenyl)-propionitrile -DPN (10 nmol/l) on Na^+-dependent Ca^{2+} efflux were measured by incubating Ca^{2+}-preloaded mitochondria with ICI 182,780 for 20 min or PPT and DPN for 10 min, before Na^+/EDTA efflux initiation.

Results and conclusions: In control conditions (without E2) Ca^{2+} efflux from Ca^{2+}-preloaded mitochondria was 2.26 nmol Ca^{2+}/mg protein. E2 caused about 35% mitochondrial Ca^{2+} efflux decrease, together with increased affinity of the Na^+/Ca^{2+} exchanger for Na^+. By promoting Ca^{2+} retention in mitochondria, E2 protects neural cells from Ca^{2+} overloading. The neuroprotective E2 action was enabled by ERα and ERβ, which is confirmed by mitochondrial Ca^{2+} efflux decrement in presence of DPN (22%) and PPT (12%). The presence of ERα and Erβ was detected by immunoblot. However, the involvement of mitochondrial E2 specific binding sites different from ERα and ERβ should not be excluded because Ca^{2+} efflux was decreased (by 14%) in the presence of ICI 182,780. Our results highlight the pathways through which E2 regulates mitochondrial Ca^{2+} sequestration and consequently protects global cell Ca^{2+} homeostasis in brain steam.

Impact of intracranial blood flow redistribution on stroke size during ischemia-reperfusion in 7-day-old rats

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Objective: Animal models have been developed to understand the pathophysiological mechanisms underlying ischemic disease and to study neuroprotection. Nevertheless, these models produce heterogeneous lesion volumes including animals without lesion. We hypothesized that the absence of cerebral lesion could be partly explained by the opening of the intracranial arterial collaterality through the circle of Willis and/or through the cortical anastomosis between the vascular beds of the three terminal cerebral arteries (anterior, middle, posterior cerebral arteries).

Material and methods: Ischemia was performed in Wistar P7 rats [2]. Briefly, anesthetized rats were exposed to left middle cerebral artery electocoagulation (MCAo) followed by a 50 minutes occlusion of either the left common carotid artery (first sets of experiments, I/R-1, $n = 68$) or both common carotid arteries (second set of experiments, I/R-2, $n = 30$). Blood flow velocities (BFV) were measured, in the internal carotid arteries and basilar trunk upstream the circle of Willis, and in the posterior cerebral arteries downstream 1) before, 2) during ischemia, and 3) after release of CCA(s) occlusion using an echocardiograph (Vivid 7, GE Medical Systems ultrasound®, Horten, Norway) equipped with a 12-MHz linear transducer (12L) as previously reported [1]. Cortical regional cerebral blood flow (rCBF) was monitored in the MCA territory by laser Doppler flowmetry. Lesion volumes were evaluated at 48 hours post-injury on cresyl violet-stained sections.

Results: At 48 hours after ischemia 41 to 48% (I/R-1 model) and 30% (I/R-2 model) of rats did not present a lesion. Those rats displayed increased mean BFV in both right internal carotid artery and basilar trunk in I/R-1 model, and increased mean BFV in the basilar trunk (BT) in I/R-2 model. In contrast, no significant changes in mean BFV were observed in lesioned rats. Furthermore, mean BFV in the BT was inversely correlated to the size of the lesion ($R^2 = 0.72$, $p < 0.0001$) in the I/R-2 model.

Conclusions: We demonstrated the protective role of collateral flow in P7 rats. Ultrasound imaging points it out and predicts absence or presence of ischemic lesions. This novel approach should greatly help preclinical studies.

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In vivo real time imaging of t lymphocytes in mouse brain by multiphoton microscopy after permanent middle cerebral artery occlusion

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Objective: Blood-brain barrier breakdown after stroke allows release of CNS antigens, and active immune tolerance to CNS antigens can decrease infarct size, presumably via the interaction of infiltrated T cells with local antigen-presenting cells. We are investigating possible roles of T cells in stroke by visualising movements of infiltrated T cells in the brain *in vivo* in transgenic mice whose T cells express GFP under the hCD2 promoter.

Material and methods: Mice underwent distal permanent middle cerebral artery occlusion (pMCAo) by electrocoagulation. Blood vessels were labelled with a red fluorescent marker (rhodamine isothiocyanate-dextran, or coated quantum dots) and the skull was thinned over an area including MCA territory. Blood vessels were imaged to a depth of about 150mm below the thinned skull by multiphoton microscopy in isoflurane anaesthetized mice.

Results: The green fluorescent T cells we tracked were associated with pial blood vessels. At 72 h after sham surgery there was a small population of infiltrated T cells close under the skull (no more than 2 cells per imaging volume of 284 mm $\times$ 284mm $\times$ 20 mm). Most of these cells meandered slowly (0.03 mm/sec) on the abluminal surfaces of blood vessels, with total displacement $<$ 5 mm in 10 min. In pMCAo mice, at 72 h, there were in the order of ten times more infiltrated T cells, associated with both well perfused and poorly perfused blood vessels (but absent from ischemic core). Their velocity distribution had two major peaks at about 0.03 and 0.14 mm/sec. The great majority meandered and had a low displacement rate (0–0.12 mm/sec), but a small minority went further (e.g. 180 mm in 10 min) and sometimes moved from the abluminal surface of one vessel to another.

Conclusion: We have visualised *in vivo* for the first time T cell behaviour after cerebral ischaemia. Our next step is to define the cellular interactions of T cells and understand how manipulating these interactions affects stroke pathology with a view to future therapeutic exploitation.

Multi-modal imaging of acute ischemic stroke: Focus on spreading depolarisations

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Ischemic stroke occurs when emboli or local thrombotic processes occlude a major brain artery. The resulting gradual blood flow decline in the territory of such artery leads to an early development of three tissue compartments: i) the ischemic core that deteriorates very fast and is not accessible to treatment if reperfusion is not started within a very short time period after the stroke; ii) the border zone around the core, the ischemic penumbra, with preserved cell integrity but disturbed function due to moderately decreased blood flow and a better chance to recover functionally, if blood flow is restored; and iii) an oligemic zone around the penumbra with mildly reduced blood flow but preserved function that may deteriorate over time, if blood flow does not recover and the inner two zones of the ischemic territory expand into the outer zones.

A complex cascade of pathophysiological processes evolving in these compartments contributes to progressive injury. Experimental studies using quantitative positron emission tomography (PET) have shown that in the acute stage, local tissue fate depends both on the severity and on the duration of regional blood flow reduction, and that effective reperfusion can be achieved only for limited time periods. In patients, pathophysiological heterogeneity makes fast, imaging-based diagnosis and therapy decision indispensable. Diffusion- and perfusion-weighted MR (DWI-PWI) and CT-based perfusion imaging are more and more used to decide on thrombolytic treatment, and validated against PET.

PET allows comparing regional cerebral blood flow with other parameters like oxygen or glucose consumption. It allows also studying in the subacute phase of stroke delayed mechanisms of damage like apoptosis targeting molecular alterations related for example to activation of caspases or inflammation targeting expression of peripheral benzodiazepine receptors on resident microglia.

As another mechanism of delayed damage, transient spreading depolarisations emerge spontaneously at the border of the ischemic core and propagate over cerebral cortex. The number of these depolarisations determines infarct size, but until recently, the basis of this relationship has remained unclear. Real-time imaging using Laser Speckle Flowmetry has demonstrated that waves of such depolarisations cycle repeatedly around the whole perimeter of ischemic lesions, resulting in regular periodicity and enlarging the lesion with each cycle. Evidence exists now from clinical monitoring to suggest that depolarisations behave similarly in the ischemic human brain. Interestingly, these propagating depolarisations are known to induce apoptotic and inflammatory processes.

Future investigations will have to focus on causal relationships between the various mechanisms of delayed damage using multimodal imaging approaches to form a better basis for new therapeutic approaches targeted at secondary brain injury.

Assessment of plasticity in the human brain

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While previously the brain was thought to be similar to a hard-wired computing apparatus which runs "software" to meet certain tasks, it has become clear in the last few decades that the brain is continuously changing its structure and function in response to inputs from inside and outside the body: "*We never use the same brain twice*". Early studies on brain plasticity were limited to invasive animal studies, however, in recent years, new neuroimaging approaches have made it possible to study plasticity processes in the human brain noninvasively.

We study brain plasticity in healthy subjects and in patients after stroke. Our focus is on the sensorimotor system. Plasticity is induced by procedural learning tasks, biofeedback and various forms of peripheral and central stimulation approaches. Structural changes in gray matter are identified with magnetic resonance based voxel-based morphometry (VBM), changes in white matter with diffusion tensor imaging (DTI), functional magnetic resonance imaging (fMRI) offers the possibility of showing changes in activity patterns during performance of tasks while functional connectivity brain imaging (fc-fMRI) identifies alterations in network organization.

In a recent study, we compared the relationship of the above mentioned different noninvasive measures of brain plasticity during procedural learning. Healthy subjects had to learn a balancing task during several weeks of training. Changes in gray matter density of premotor cortex were notable already after one week, however, despite continuous training and further functional improvement these changes tended to disappear thereafter. On the other hand, changes in gray matter density in prefrontal areas developed with a slower time course and matched improvements in performance, i.e. were persistent with permanent improvement in function. Structural changes in white matter closely matched those in gray matter. Furthermore, the same areas in which structural changes were noted were also part of a network in which functional connectivity changed with improved performance. Thus, this study illustrates that improved function during learning is accompanied by separable phases of structural and functional changes in different parts of a brain network. It also illustrates that a comprehensive "picture" of brain plasticity can only be obtained by including not just one but several of the above mentioned noninvasive neuroimaging approaches.

Magnetic- and optogenetic methods for stem cell tracking and control in the CNS

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Various adult stem cell populations are currently being evaluated regarding their restorative potential in the experimental therapy of degenerative CNS disorders. In previous studies we were able to visualize transplanted stem cells by high resolution MRI *in vivo*, by labeling the cells with iron-oxide nanoparticles (VSOP). However, the key question whether this effect is mediated by restoring network function remains open up to now, as probing of newly generated stem-cell derived neurons *in vivo* has not been feasible. The discovery of a rapidly gated light-sensitive cation channel channelrhodopsin-2 (ChR2) suitable for noninvasive control of neuronal activity has made it possible to optically control membrane depolarization on the millisecond timescale. We introduce ChR2 into embryonic stem cells and develop optogenetic technology for stem cell engineering applications. Mouse embryonic

stem cells (ESCs) were stably transduced with ChR2-YFP. Illumination of resulting ChR2-ESCs with pulses of blue light triggered strong inward currents. These labeled ESCs retained the capability to differentiate into functional mature neurons, assessed by the presence of voltage-gated sodium currents, action potentials, fast excitatory synaptic transmission, and expression of mature neuronal proteins and morphology. Optically stimulating ChR2-ESCs during the first 5 days of neuronal differentiation, with high-speed optical switching on a custom robotic stage and environmental chamber for integrated optical stimulation and automated imaging, drove increased expression of neural markers. These data point to potential uses of ChR2 technology for chronic and temporally precise noninvasive optical control of embryonic stem cells both *in vitro* and *in vivo*, ranging from noninvasive control of stem cell differentiation to causal assessment of the specific contribution of transplanted cells to tissue and network function.

New directions in MRI of the brain

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There continues to be rapid development of MRI to image the brain both in humans and pre-clinical animal models. Over the past few years work from our laboratory has focused on extending MRI to detect cytoarchitecture at the level of specific layers, push the spatial limits of functional MRI, and develop tools to monitor migration of single endogenous progenitor cells in the rodent brain. Work from these areas will be presented.

MRI to detect cytoarchitecture can now be accoplished using three contrast mechanisms. T1 weighted MRI detects myelination in cortex, phase sensitve susceptibility weighting detects primarily iron and gives robust cytoarchitectural definition in human and manganese enhanced MRI allows delineation of a number of brain areas in animals. Extending contrast and resolution in MRI should lead to an unprecedented ability to quantitate brain areas *in vivo*.

The spatial and temporal limits of fMRI have not been fully defined. Recently we have been extending fMRI to determine the mechanism for circuit changes that underlie large scale cortial plasticity that can occur after nerve damage. This work has required trying to clearly define functional boundaries as well as detemine with laminar specificity where synaptic changes that underlie the plasticity occur. Application to a model of cortical plasticity induced by peripheral nerve injury will be described.

There continues to be great interest in using progenitor cells to enhance recovery of the brain. Key to this will be development of non-invasive imaging techniques that enable monitoring cell migration, differentiation and integration. Recently we have demonstrated that micron sized particles of iron oxide can be used to label endogenous precursor cells and enable MRI to monitor their migration and integration into the olfactory bulb. The effects of presenting naive animals with odor on migration have been assessed.

The prospects for translation of each of these MRI techniques to study neural injury and repair will be discussed.

Mild ischeamia causes rapid disruption of axon-glial integrity and cognitive dysfunction

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Ageing of the brain can lead to cognitive decline which causes severe personal and social problems. Studies have shown that with advancing age there is a decline in white matter integrity and changes in myelin structure and function are suggested to contribute to cognitive decline. The occurrence of changes in white matter are often linked with chronic cerebral hypoperfusion (modest reductions in cerebral blood flow). In humans investigating the causes of these changes is strictly limited to brain imaging and in some cases post-mortem tissue analysis. Thus the underlying morphological changes and cellular mechanisms in white matter remain largely unclear in the ageing brain.

A mouse model of chronic cerebral hypoperfusion has been developed, whereby hypoperfusion is induced by application of microcoils to both common carotid arteries (BCAS). In this BCAS model, incredibly modest reductions in blood flow results in selective damage to the white matter and mimics aspects of cerebrovascular white matter lesions. This model permits insight to mechanisms that occur early in response to hypoperfusion that are prohibited in human brain.

Here we investigated the effects of chronic cerebral hypoperfusion on the integrity of the white matter at the cellular level using detailed confocal imaging. Key

proteins were analysed at the axon-glial junction after chronic cerebral hypoperfusion. We found that the axon-glial connection is disrupted early in response to BCAS (within 3 days) in the absence of major changes to the myelin and axon. Furthermore, at later times after BCAS (1 month) these white matter changes were associated with a cognitive impairment, specifically working memory. Together the results highlight the vulnerability of key components of white matter to modest reductions in blood flow which is sufficient to impair cognitive abilities.

This work was conducted as part of the Disconnected Mind program, supported by Age Concern.

Translational stroke research

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Translational research represents the confluence between basic and clinical science. Typically, translation occurs when a basic science advance occurs and clinical relevance for this advance is sought. For example, the discovery of a new pathway of ischemic cell death leads to the development of molecules targeted to interfere with this purported contributor to ischemic injury. After basic pharmacology and toxicology are performed, the potential therapeutic agent is evaluated in relevant animal stroke models. Protective molecules that show initial promise can then be more rigorously assessed by animal models that incorporate advanced MRI techniques such as diffusion/perfusion MRI to precisely evaluate its effects on evolution of the ischemic penumbra and also its pharmacodynamics. These advanced MRI techniques are truly translational because very similar imaging can be performed in stroke patients to confirm similar effects of novel therapeutic agents. The last step in the translation of promising novel molecules is the clinical trial program that tests safety, proof of concept that the new drug salvages ischemic tissue and ultimately in phase III hopefully demonstrates significant clinical efficacy. The translational pathway can also occur in the opposite direction. A clinical phenomenon can be explored by basic scientists in an attempt to elucidate the mechanisms related to a clinically relevant problem. For example, after tPA was shown to be effective in improving stroke outcome concerns arose about potential toxicity and this was confirmed by basic research. A third approach would be collateral translational research. Collateral research would be exemplified by laboratory attempts to develop improved thrombolytic agents or combinations of agents to improve recanalization/reperfusion with a better safety profile. An example of this translational approach would be the combined use of annexi-2 with tPA, a combination with apparent better lytic efficacy and safety than standard tPA

Neuroprotective therapy for ischemic stroke with free radical scavenger edaravone, gene-stem cell therapy, and in vivo optical imaging of ischemic brain damage

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As well as blood flow restoration, neuroprotection is essential for therapy in acute stage of stroke. Both NTFs and free radical scavenger can be such neuroprotective reagents with inhibiting death signals and potentiating survival signals under cerebral ischemia. For example, topical application of GDNF greatly reduced the infarct size and brain edema after middle cerebral artery (MCA) occlusion in rats. The reduction of the infarct size was not related to a change of cerebral blood flow (CBF), but was accompanied by marked reduction of positive cells for TUNEL and caspases in the affected area. Thus, GDNF showed a direct protective effect against ischemic brain damage, but not secondary by improving CBF. Sendai virus vectors containing the GDNF gene showed a great reduction of infract volume without affecting regional CBF but with reducing translocation of apoptosis inducible factor (AIF) from mitochondria to cytoplasm.

A free radical scavenger Edaravone is the first clinical drug for neuroprotection in the world which has been used from 2001 in most ischemic stroke patients in Japan. Edaravone scavenges hydroxyl radicals both in hydrophilic and hydrophobic conditions, and is especially useful in thrombolytic therapy with tissue plasminogen activator (tPA). Combination therapy of Edaravone with tPA greatly increased survival of stroke animals, reduced infarct size, and inhibited molecular markers of oxidative damage in lipid, protein and DNA. Use of Edaravone greatly reduced hemorrhagic transformation accompanied by tPA treatment,and may also extend therapeutic time window with tPA therapy for more than 3 hr in human stroke patients.

Of great importance for regenerative therapy are the neural stem cells which are intrinsically activated or

exogenously transplanted. To support stem cell migration, an artificial scaffold can be implanted to injured brain for promoting ischemic brain repair. Addition of NTFs greatly enhanced an intrinsic migration or invasion of stem cells into the scaffold, which could provide a future regenerative potential against ischemic brain damage at chronic stage.

In vivo optical imaging of cerebral ischemic injury may provide a new approach to diagnose and treat patients of ischemic stroke. Our recent results show that it is possible to detect an autophagic process, an early apoptosis, and activation of MMP-9 in living mice brain, which may be ameliorated by some neuroprotective therapies.

Stem cell models of neurodegenerative disease for drug discovery

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Question: Microglial cells have a causal role in amyotrophic lateral sclerosis (ALS) and possibly many other neurodegenerative diseases. Chronic activation of microglial cells is neurotoxic and is a hallmark of ALS. Inhibition of microglial activation and toxicity leads to extended survival and better clinical outcome. Therefore, microglial activation and toxicity are attractive targets for drug discovery. We have harnessed the properties of stem cells to create an *in-vitro* model of microglial toxicity for high-throughput screening.

Methods: Cultures of mouse ES cell derived motor neurons, mouse neural stem cell derived astrocytes and activated microglia were integrated. Using GFP and high content imaging, an assay platform for microglial toxicity was established. A pilot screen of libraries of approved drugs identified was conducted.

Results and conclusions: Several compounds that signficantly inhibited microglial toxicity were identified. Secondary assays demonstrated that the active compounds act through a vareity of different mechanisms. Our work provides proof-of-principle for stem cell-based drug discovery.

iPS for stroke

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The possibility to reprogram somatic cells into embryonic stem cell(ESC)-like pluripotent stem cells, the iPSC, has generated unprecedent hopes for stem cell therapy in a number of diseases with unmet medical need. Stem cell therapy includes regenerative medicine with replacement of lost cells and improvement of the host brain plasticity through a still ill-defined array of actions.

IPSC display the advantages of ESC, i.e. illimited self-renewal and pluripotency, which are main assets for stem cell therapy in acute brain lesions. However, self-renewal and pluripotency properties, together with potential re-expression of the transgenes used for iPSC derivation, introduce significant challenges for clinical applications. This study aimed at investigating gender, safety, and efficacy aspects of human (h)iPSC therapy in stroke.

Methods: Two hiPSC lines (XX and XY, named ShiPS) were derived from human fibroblasts (IMR90, XX, MRC5, XY, ATCC) with lentivirally-mediated expression of OCT4, SOX2, NANOG and LIN28. Homogenous pools of neuronal precursors (NPC) were derived and assessed for lack of expression of pluripotency genes. They were grafted into the lesion cavity seven days after 90 min occlusion of the middle cerebral artery (MCAO) in male and female rats. Comparisons were made with the SA001 (XY) and CCTL14 (XX) hESC lines. Motor, locomotor and cognitive impairments were monitored over four months with an extended set of tests. Grafts were followed by T2*-weighted MRI over the 4 months. Imaging data were correlated post-mortem with histological observations. Animals were either immunosuppressed with cyclosporin A or immunotolerized at birth with the cells-to-be-grafted. The later allowed grafting in both males and females littermates to approach gender issues.

Results: All cell types were well tolerized and overall graft survival was of 72%. No significant differences were observed between male and female cells in terms of differentiation and integration into the host brain. Terminal differentiation into striatal DARPP-32 medium-spiny GABAergic neurons was observed with all progenitors, although with a slower timing with hiPS-derived neural progenitors. Cells migrated into the host brain with preferential direction towards the lesion. In all cases, the host brain provided the grafts

with astrocytes and a conspicuous vascularisation. Behavioural studies revealed a rapid spontaneous recovery of motor and sensory-motor deficits animals with lesions restricted to the striatum, with no differences between grafted or control animals. In animals with large lesions encompassing both the striatum and cortex, grafting significantly improved the recovery, with no differences for host gender or cell types.

In conclusion, the ShiPS lines appeared indistinguishable from hESC in terms of their differentiation properties and behavior after NPC transplantation into the post-ischemic brain. This study provides pre-clinical evidence that human iPSC-derived NPC represent a safe and effective source for regenerative medicine in stroke, independent of gender.

This work was performed within the STEMS (*Preclinical analysis of stem cell therapy for stroke*) *European consortium* (*www.stemsproject.eu*) *with additional grants from the Association Française contre les Myopathies and the grant 1M0538 from the Ministry of Education, Youth and Sports of the Czech Republic.*

Human stem cell therapy for stroke

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Human stem cell transplantation is emerging as a promising therapy for restoring function after experimental stroke. This talk will review the (1) therapeutic benefit of human fetal and embryonic derived stem cells in various rodent stroke models; (2) potential molecular and cellular mechanisms underlying functional recovery following transplantation; (3) completed clinical stem cell transplant studies for stroke; (4) use of imaging modalities to monitor cell therapy; and (5) unresolved issues confronting successful translation of experimental stroke cellular transplant research into future clinical applications.

Clinical translation of stem cells for brain repair following stroke

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Transplanted cells of different sources have induced some improvement in animals and humans affected with stroke. It is important to emphasize, though, that improvements after cell implantation into the injured brain can be explained by several different mechanisms, such as inflammation and release of trophic molecules, leading to neuroprotection and sprouting, and re-establishment of a local reinnervation of the denervated area. For stem cell therapy to be of major clinical value in stroke, it is highly likely that cells of human origin should be able to replace dead neurons and repair damaged neural circuitries. Several studies have now been performed in which human neural stem cells (NSCs) as well as stem cells from other tissues have been delivered in animal models of stroke. The current status in this field will be reviewed. Experimental evidence obtained mainly in rodents has indicated that the stroke-damaged adult brain also makes an attempt to repair itself by producing new neurons from its own neural stem cells. During several months after stroke, NSCs in the subventricular zone generated new striatal neurons which migrated to damaged area. Currently, our knowledge about the mechanisms regulating the different steps of endogenous neurogenesis after stroke is incomplete. It is highly likely that in order to have a substantial impact on the recovery after stroke, neurogenesis has to be markedly enhanced. Based on available data this should primarily be achieved by increasing the survival and differentiation of the generated neuroblasts. Moreover, for maximum functional recovery, optimization of endogenous neurogenesis most likely needs to be combined with stimulation of other neuroregenerative responses, e.g., protection and sprouting of remaining mature neurons, and the transplantation of stem cell-derived neurons and glia cells.

Cell therapy of acute ischemic stroke

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There is only one U.S. Food and Drug Administration (FDA)-approved treatment for acute ischemic stroke, tissue plasminogen activator (tPA). However, no other neuroprotective trials have been positive. There is growing interest in neurorestorative treatments that can improve functional outcome and enhance brain plasticity and that can be administered after 24 hours. Proposed restorative therapies include growth factors, Phosphodiesterase 5A inhibitors, and cell therapy.

We propose that there are two time windows of intervention for cell therapy after stroke: a.) “subacute”

in the 24 hour to week period where there is active brain remodeling and many available targets and b.) a late or "chronic" period (after 3–6 months) after maximal recovery has occurred but complicated by gliosis and scar. The routes of transplantation include direct intracerebral, intranasal, intra-arterial, or intravenous. There is growing evidence that intravenous delivered stem cells migrate to the spleen where they exert immunomodulatory effects and the lung where they secrete anti-inflammatory factors. Allogeneic intravascular therapies are attractive as they would offer an "off the shelf" product that would be more "scalable" than an autologous therapy. The recent discovery of induced pluripotent stem cells (iPS) offers another potential restorative therapy. It will be important to carefully design clinical trials to avoid complications and setbacks that have hampered the gene therapy field.

Role of adiponectin receptors in ischaemic stroke induced neuronal cell death

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Objective: Adiponectin is a 244 amino acid adipokine found in peripheral circulation as a cleaved globular fraction and multimers of a full length monomer. These different isoforms exert various actions peripherally by activating different signaling molecules like adenosine monophosphate-activated protein kinase (AMPK), p38 mitogen activated protein kinase (p38MAPK), peroxisome proliferator activated receptor α (PPARα) and nuclear factor-kappa B (NFkB) transcription factors, through two transmembrane receptors, adiponectin receptor 1 and 2 (ADR1, ADR2). Recent studies on adiponectin have warranted the possibility that adiponectin can exert anti-inflammatory or pro-inflammatory actions, depending on the biological milieu the isoforms are subjected to and the type of ADR receptors (ADR1 or ADR2) they activate. The presence of adiponectin receptors (ADRs) on mice hypothalamic neurons invoked our hypothesis of primary cortical neurons express ADRs and activation of ADRs play a role in ischaemic stroke induced neuronal cell death. Hence, the objective of our study is to explore the presence of ADRs in cortical neurons and the effect of their activation in stroke.

Material and methods: By using multiple techniques such as PCR, immunoblot and Immunocytochemistry, we studied the presence and function of ADRs in neuron following ischaemic stroke conditions.

Results and conclusion: We found that primary mouse cortical neurons express ADR 1 and ADR 2. Furthermore, immunoblot analysis confirmed that levels of ADR1 and ADR2 were increased within 3–18 h of the onset of oxygen and glucose deprived (OGD) condition with ADR1 expression more elevated as compared to ADR2. In order to see the expression of ADRs *in vivo*, ipsilateral brain sections were analysed following cerebral ischaemia and reperfusion (I/R). In the cerebral cortex of sham-operated control mice, little or no ADR1 and ADR2 expression was observed. At 6 h after stroke, neurons in the ischaemic cortex exhibited robust ADR1 and ADR2 expression. Next we studied the effect of adiponectin treatment in cortical neuronal culture following OGD condition. We found that adiponectin mediated ADR activation increased downstream activation of various signaling pathways such as p-AMPK, and p38-MAPK that could play a role in OGD induced neuronal cell death. The identification of ADRs dependent signaling pathways in neuronal cell death and adiponection-ADRs interaction in neurons could be promising in devising a multi targeted therapeutic intervention in ischaemic stroke.

Improved functional recovery after stroke through enhancement of the endogenous neurogenesis in aged rats

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Background: In adult rats the endogenous neurogenesis is maintained in the subventricular zone and the dentate gyrus of the hippocampus and could be used to improve post-stroke outcome. Here we explored the hypothesis that stimulations of endogenous neurogenesis before or after stroke in aged rats, which are known to be more severily affected by stroke than young rats, may improve recuperation after stroke.

Methods: Stroke was induced by middle cerebral artery occlusion (MCAO) in aged rats and neurogenesis was stimulated at different time points using neurogenesis enhancer, pentylentetrazole.. After MCAO, rats

were behaviorally tested for 7 weeks. After 7 weeks, global gene expression analyses of the periinfacted region was done.

Results and conclusion: Behavioral testing by T-Maze (labyrinth) and inclined plane tests showed improved rehabilitation in rats with a stimulated neurogenesis after stroke. Immunhistochemical stainings for doublecortin, a marker of neurogenesis, showed increased expression in rats stimulated after, and to a lesser extent after stroke. Global gene expression analysis revealed that most of the signalling pathways implicated in neurogenesis are downregulated in both age groups.. Our results indicate that stimulation of neurogenesis at 4 wks before stroke does not improve post-stroke outcome. In contrast, stimulation of post-stroke neurogenesis is beneficial for behavioral recovery of aged rats.

Placental expanded (PLX) cells attenuate inflammatory and neuropathic pain

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PLX are stromal cells derived from human placenta and expanded in a three-dimensional culture. These cells express CD29, CD73, CD105 but not CD14, CD45, CD31 and HLA-DR. In addition, PLX cells bear the capacity of reducing inflammation, inhibiting T cell proliferation and promoting angiogenesis, most likely, via a paracrine signaling through cytokine secretion. Recently, intramuscular administration of PLX cells to critical limb ischemia patients has been shown to be safe in two phase I clinical studies performed in parallel in the EU and the US. Here we hypothesized that PLX cells improve inflammatory and neuropathic pain, representing prevalent and difficult to treat forms of chronic pathological pain. As models of such conditions we used a complete Freund's adjuvant (CFA)-induced inflammation of the rat hind paw and a chronic constriction injury of the sciatic nerve (CCI) in mice, respectively. *In vitro* it was observed that PLX cells contain opioid peptides, dynorphin A, enkephalin and beta-endorphin, as measured by ELISA. *In vivo*, PLX cells were injected into inflamed rat paws or at the site of nerve injury in mice, two days after CCI or CFA injection. Pain was measured by paw pressure, von Frey and Hargreaves tests. Treatment with PLX cells resulted in attenuation of both mechanical and thermal hypersensitivity that lasted 4 days after their injection, in both inflammatory and neuropathic pain. Our study shows that PLX cells express opioid peptides and when injected into injured tissues can ameliorate pain. Actions of currently used medications for chronic pain are hampered by serious adverse effects such as addiction, sedation, gastrointestinal ulcers and bleeding, kidney and liver toxicity and cognitive impairment. Our findings offer PLX cell-based therapy as a novel strategy for promising management of painful inflammatory and neuropathic conditions.

Immunological profile of Critical Limb Ischemia patients Following intramuscular administration of Placenta ADHERENT stromal cells

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Cell therapy for critical limb ischemia (CLI) offers an opportunity for patients that have exhausted all currently available interventions. Two phase I open-label, dose-escalation studies, intended for the treatment of CLI were initiated in parallel in the EU and U.S. Enrollment was completed in Germany, and is ongoing in the US. Cells were administered in 30–50 intramuscularly injections in the effected limb. Five dosing groups are being evaluated reaching a maximum dose of 560×10^6 cells. Due to the allogeneic source of the PLX-PAD cells, it is imperative to evaluate each patient for both humoral and cellular immune responses to the allogeneic HLA type. We further test for different criteria of immunosuppression of lymphocytes and monocytes, and the level of pro and anti-inflammatory cytokines in the blood following *ex-vivo* stimulation of PBMCs. In addition, the study patients are tested for endothelial cell activation and for the level of immune responses to latent pathogens. Preliminary results reported no significant adverse effects related to PLX-PAD administration. Analysis related to twenty one patients who have completed their three-month follow-up is available. The immunological tests for the low and intermediate doses will be presented. Our data is the first extensive immunological evaluation of any mesenchymal-like cell therapy in clinical trials of CLI. Moreover, such data will have broad implications for mesenchymal-like cell therapy in other disorders.

Adipose derived stem and regenerative cells for ischemic diseases

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Adipose Derived Regenerative Cells (ADRCs) are a heterogenous group of cells derived from liposuctioned adipose tissue. This abundant cell population exhibits characteristics which may mitigate the damage caused by hypoxia. In addition, ADRCs can be readily obtained at the bedside within a clinically relevant period of time. Thus, they are an increasingly being considered as treatments for ischemic conditions ranging from subcuatneous wounds to myocardial infarction.

Perlecan Domain V generation and therapeutic potential in focal cerebral ischemia

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Stroke is a significant cause of death and serious long-term disability. However, therapeutic advances have been infrequent due in part to a poor understanding of the mechanisms of post-stroke brain repair, which involve neurovascular coupling of angiogenesis and neurogenesis. These processes also involve extracellular matrix (ECM) remodeling and generation of ECM fragments. We sought to demonstrate whether one of these ECM fragments, perlecan domain V (DV), could affect post-stroke brain repair. Our objectives were based on previous observations that ischemic stroke rapidly generates fragments of perlecan, and perlecan is required for angiogenesis and neurogenesis. Animals underwent left MCA occlusive stroke via stereotactic injections of endothelin-1 (rats) or via physical MCA occlusion (mice) followed by intraperitoneal injections of DV (0.5–1 mg/kg) or PBS control on post-stroke days 1, 3, 5 and 7. Motor function was assessed via the vibrissae elicited stepping reflex (mice), or cylinder test (rats). Additionally, the potential effects of DV on angiogenesis and neurogenesis were studied *in vitro* with isolated rodent brain microvascular endothelial cells. We first noted that DV was rapidly generated after stroke and persisted for 7 days. From post-stroke day 3 onward, stroked rats and mice treated with DV were statistically indistinguishable from sham surgery controls in motor function, while untreated stroked animals remained significantly impaired throughout testing. DV specifically homed to injured brain and increased peri-infarct angiogenesis and neurogenesis. Surprisingly, DV also was neuroprotective in that initial stroke volumes were smaller and there were fewer apoptotic peri-infarct neurons with DV treatment. *In vitro* studies further demonstrated that this occurred in part by causing endothelial cell release of VEGF. Therefore, in two distinct stroke animal models, perlecan DV homes to stroked brain tissue and improves functional stroke outcome by enhancing post-stroke angiogenesis and being neuroprotective. Our results suggest that DV could be a promising new stroke treatment.

PPAR as a pharmacological target for neuroprotection and neurorepair

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It remains an imperative need to development of neuroprotective agents in stroke to: (i) limit the immediate functional consequences and the long-term motor and cognitive consequences; (ii) serve as a possible adjuvant for thrombolysis by decreasing the haemorrhagic risk and extending the therapeutic window. Repeated failures of neuroprotective agents prompted the search of pharmacological strategies with pleiotropic mechanisms that could target the whole neurovascular unit. Of the pharmacological targets likely to induce a pleiotropic brain effect after stroke, peroxisome proliferator-activated receptors (PPARs) are prime candidates, in as much as they are expressed by all three compartments in the neurovascular unit and are able to modulate many molecular pathways involved in pathophysiology of stroke. Among several isoforms of nuclear receptors PPARs (alpha, beta/delta, gamma), we focused on PPARalpha isoform because its pharmacological properties on leucocyte-endothelium interactions, inflammation, stem cells and amyloid cascade. Moreover, we have long-term clinical experience on the good tolerability of fibrates, a class of lipid-lowering drugs that are synthetic activators of PPARalpha. The effect of PPAR-alpha stimulation by fibrates on brain ischaemia has mainly been studied on a pre-treatment basis. However, recent experimental results suggest that some of the effects during the preventive treatment with fibrates could result from an acute effect during the onset of ischaemia. Here, we will demonstrate that acute modulation of PPAR-alpha in animal models of brain ischaemia generates beneficial effects in term of not only the immediate post-stroke consequences but

also the longer-term processes involved in neurorepair and the pathophysiology of post-stroke dementia.

Neural repair and recovery in peri-infarct cortical circuits

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Recovery after stroke in humans is closely associated with changes in cognitive maps in peri-infarct cortex and connected cortical areas. Studies in animal models of stroke indicate that axonal sprouting and the formation of new connections also occurs in per-infarct and connected cortical areas in stroke. However, it has not been determined whether axonal sprouting in cortical circuits after stroke mediates recovery and, if so, the molecular mechanisms that underlie post-stroke axonal sprouting. We have completed a transcriptional profile of sprouting neurons in peri-infarct cortex after stroke. This data indicates a strong upregulation of EphrinA signaling both at the receptor (EphA4) and intracellular levels (α chimaerin) in sprouting neurons. Selective isolation of reactive astrocytes after stroke shows a 73x upregulation of an EphA4 ligand, ephrinA5. Local blockade of ephrinA5 signaling in peri-infarct cortex after stroke induces axonal sprouting in motor and premotor circuits, and behavioral recovery. Local induction of ephrinA5 signaling after stroke blocks axonal sprouting and blocks behavioral recovery. This data identifies a new glial growth inhibitory protein, the paradoxical upregulation of its receptor in sprouting neurons, and shows a causal role for ephrinA5 in functional recovery after stroke. Additional results from the transcriptional profile of sprouting neurons after stroke suggests growth-promoting molecules that mediate the induction of axonal sprouting and could serve as targets for the promotion of repair and recovery in this disease.

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Dampening tonic inhibition promotes post-stroke functional recovery in young and aged

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Objectives: Post-stroke neural repair and rehabilitation processes continue for weeks to months after the initial insult. Post-stroke neuronal repair involves, motor learning, synaptic plasticity, axonal sprouting, and cortical reorganization. In developing and adult animals these processes are in part mediated by activity dependent physiological changes. Extrasynaptic $GABA_A$ receptors, consisting of alpha5 ($\alpha 5$) or delta (δ) subunits, mediate a tonic form of neuronal inhibition that controls baseline levels of excitability. The current study assessed the role of tonic GABA-mediated inhibition on post-stroke recovery using pharmacogenetic manipulations to $\alpha 5$ and δ-subunit containing $GABA_A$ receptors in young and aged mice.

Materials and methods: Photothrombotic stroke was induced in mouse forelimb motor cortex of young adult (2–3-months) or aged (22–24-months) animals. Whole-cell patch-clamp electrophysiology was used to assess changes in tonic inhibition within peri-infarct layer II/III pyramidal neurons. Behavioral measures were assessed 1-week prior to stroking and then subsequently 1-, 2-, 4- and 6-weeks post-stroke on two measures of forelimb motor cortex function: rearing in the cylinder task, and accurate foot placement on the grid-walking task. L655, 708, a $GABA_A\alpha 5$ inverse agonist (2.5–5 mM) was administered via osmotic minipumps from day-3 post-stroke for the full duration of assessment (6-weeks). Western blot analyses were used to assess changes in GABA transporters, GAT-1 and -3, in peri-infarct tissue.

Results: Electrophysiology recordings of layer II/III pyramidal neurons *ex vivo* revealed an increase in tonic inhibition within the peri-infarct cortex. Application of L655,708 resulted in a decrease in peri-infarct tonic inhibition. Assessment of motor behaviors *in vivo* revealed significant ($P < 0.001$) forelimb deficits out to at least 6-weeks post-stroke. Treatment with L-655,708, starting 3-days after stroke, significantly and dose-dependently decreased forelimb deficits on both the cylinder and gridwalking tasks. These functional gains were rapid, with near maximal effects seen from 7-days post-stroke. Assessment of $\alpha 5$-/- mice showed similar functional gains of forelimb motor recovery, whilst δ-/- mice only had mild improvements. Assessment of motor function in aged mice showed greater deficits in forelimb function compared to young. Treatment with L655,708 from 3-days post-stroke resulted in

a significant decrease in forelimb deficits. These functional gains in the aged are comparable to those seen in the young. Electrophysiology and western blot analysis revealed GAT-3 down-regulation in peri-infarct cortex.

*Conclusions:*These results demonstrate that stroke produces a state of hypoexcitablity in peri-infarct motor cortex through increased tonic GABA signaling. Suppression of tonic inhibitory currents affords an early and sustained reversal of forelimb motor deficits after experimental stroke that is comparable between young and aged.

Neuronal differentiation of human iPS-cells under in vitro and in vivo conditions

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The discovery of induced pluripotent stem (iPS-) cells has opened up new vistas for regenerative medicine. In this study we have tested the neurogenic potential of human iPS cells under *in vitro* and *in vivo* conditions. For this purpose we co-cultivated neural progenitors from human iPS-cells derived fron MRC5 fibroblasts (ATCC, iPS-NP) with a primary culture from the cortex of embryonic rats containing neurons, astrocytes, oligodendrocytes and microglia. Surprisingly, already 2 days after seeding a certain number of iPS-NP exhibited a clear neuronal morphology combined with expression of betaIII-tubulin and doublecortin. In addition, we found iPS-NP without neuronal differentiation and cells already expressing betaIII-tubulin, but not having yet distinctive axonal and dendritic processes. The latter we referred to as developing neurons. iPS-NP starting neuronal differentiation, were contacted both by neuronal processes from primary neurons and by oligodendrocytes. After 7 days of co-cultivation, however, we observed that iPS-NP underwent phagocytosis by microglial cells. In addition, we transplanted the iPS-NP into rats 3 days after experimental stroke. Three month later the cells had been survived, although microglia and T-cells were attracted in a great number. Further, near to the graft blood vessels were located and neuronal differentiation could be demonstrated by DCX and ßIII-tubulin expression.

Long-term survival of human neural stem cells in the ischemic rat brain upon transient immunosuppression

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Neural stem cells (NSCs) are of considerable importance for cell-replacement therapy for neurodegenerative diseases, however, the limited supply of primary NSCs hampers the large-scale therapeutic application. Here, we investigated the fate of immortalized human neural stem cell line (IhNSC) derived from the telencephalic-diencephalic region of fetal brain, following unilateral implantation into the corpus callosum or the hippocampal fissure proximally to CA1 layer of adult rat brains after global ischemic injury We evaluated IhNSC integration and maturation in a pathological setting mimicking the chronic impairment of neurological function in acclaimed AD, and documented their ability to engraft efficiently to the point of establishing synaptic contacts with the host cells. The salient findings of this investigation are the following: 1) IhNSC transplanted *in vivo* in damaged rat brains survive, integrate (Fig. 1) and differentiate into mature neuronal cells bearing GABAergic and GLUTAmatergic phenotypes after transplantation into the damaged brain of adult rodents 2) Implanted IhNSCs are poorly immunogenic. Although several studies have shown a very low immunogenic response to transplanted NSCs, this issue has not been elucidated exhaustively. Here, we tested the grafting ability of NSC by transplantation next to the main sites of ischemic injury, characterized by strong inflammation, and demonstrate that a transient immunosuppresion is sufficient for the engraftment of the implanted IhNSCs. This finding in rats leads to extrapolate that continuous immunosuppressive therapies would not be required in patients receiving intracerebral transplantation of human NSCs. 3) A paramount issue in cell replacement therapy for CNS degenerative disorders is the ability of transplanted cell to establish synaptic contacts with host cells. We demonstrate for the first time, using electron microscopy analyses, that IhNSC-derived neurons integrate into the lesioned hippocampus as mature cells, establishing synaptic junctions with the host cells, 4

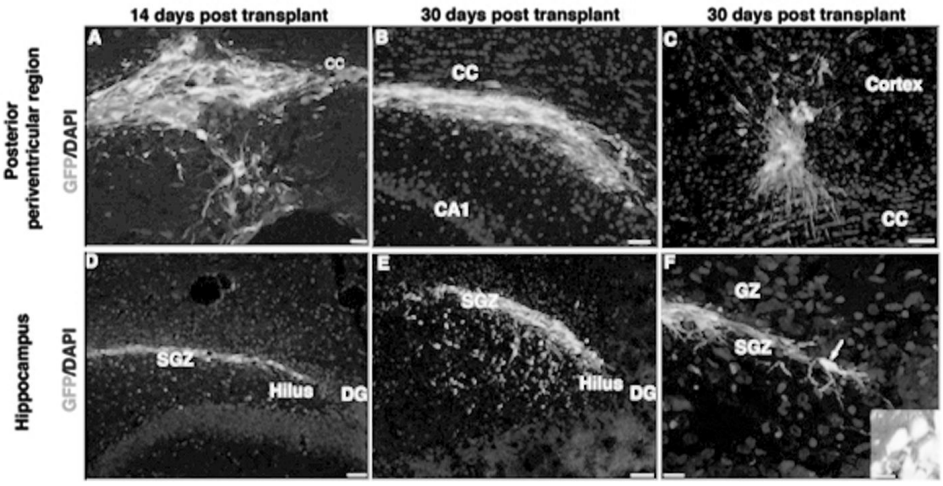

Fig. 1.

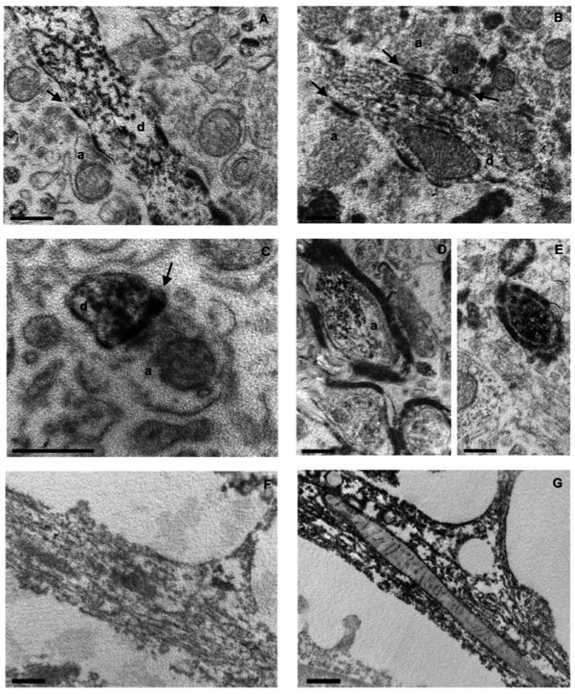

Fig. 2.

months after transplantation (Fig. 2). 4) Recent studies have elucidated immunomodulation as one of the most important therapeutic potentials of NSC in neurodegenerative diseases. In this study, we have shown that in a global inflammatory environment generated by a lesion such as the global ischemia, hNSC are able to decrease both the reactive microglial and astroglial cells, thus contributing to the endogenous recovery of the lesion. These results point to hNSC as a reliable source of cells for transplantation in patients affected by neurodegenerative diseases, such as AD, ischemia and, perhaps post-traumatic brain injuries characterized by acute inflammmatory processes, like stroke where they are also able to decrease reactive micro- and macroglial cells.

Predictive experimental model of long-term cerebral ischemic damage and neurologic outcome on rat mcao model

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Objectives: Stroke is the main cause of disability in modern society. Most stroke patients show some, albeit variable, functional recovery over time. Adult mammalian brain is able to structural reorganization after injury but these mechanisms occur over weeks and months. The aim of our study is to target time-dependent mechanisms (until 6 months reperfusion) to evidence different therapeutic targets over time for an investigation of pleiotropic or combined therapeutics.

Methods: We designed a battery of behavioral tests (motor, sensorimotor and mnesic) to explore different capacities of cerebral tissue. This behavioral investigation is correlated with magnetic resonance imaging (MRI) study (7 Tesla anatomic sequence T2) with the

middle cerebral artery occlusion rat model. Rats were submitted to 1 hour occlusion and sacrified at 28 days, 2, 4 and 6 months after the realization of behavioral tests. MRI experiments are performed at 24 h, 7 days, 28 days, 2, 4 and 6 months to assess lesion evolution, the infarct and edema volumes, hypointensity and hyperintensity signals, the atrophy of hippocampus and cortex, the development of porencephalic cysts.

Results: MRI results show the edema reduction during the first week and the progressive transformation of the lesioned tissue. In 60% of rats, hyperintensity signal evolved in porencephalic cysts as soon as 1 month reperfusion ($30+/-6$ mm3) and progressively increased to $126+/-32$ mm3 at 6 months reperfusion. The size of porencephalic cysts can be predicted by the total infarct volume at 24 hours and 7 days reperfusion. In parallel we observed a cortical atrophy of the ipsilateral hemisphere. In addition, an hypointensity signal which appeared at 7 days of reperfusion. Moreover, hippocampic atrophy appeared ipsilateral to the lesion after 2 months reperfusion. Behavioral study evidenced functional and memory deficits. Staircase test showed a sensorimotor deficit of the contralateral forepaw from 1 month reperfusion until 4 months. Actimetry test revealed an hypoactivity of ischemic rats compared to sham animals at one month reperfusion contrary to the hyperactivity observed since 4 months. Concerning mnesic activity, the spontaneous alternation test demonstrated a deficit since 4 months reperfusion.

Conclusion: These results evidenced that a tissue reorganization of ischemic region occurred at long-term reperfusion times. In parallel, behavior tests performances revealed a motor deficit followed by a recovery process in contrary to the coming out of a decrease of short-term mnesic capacity. Next step is now to confirm by immunohistochemical staining the potential degenerative and/or plastic processes involved in the aim to evidence some therapeutic targets.

Neuroprotective role of connexins in the central nervous system

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Objective: Astrocytes are a major cell type in the central nervous system and play an important role in regulating brain metabolism. Moreover, astrocytes compose the frame network and communicate through gap junctions mainly composed by connexin 43 (Cx43) subtype. We have been reported that astrocytic Cx43 may play a critical role in controlling neuronal apoptosis and inflammatory response following brain ischemia. Of course, the effects of astrocytic Cx43 are still being debated on pathological conditions. Recently, gap junctions composed of different types of connexins have been reported to have permeable selectivity to different biological molecules. A few reports have also reported alterations of the connexin expressions under pathological conditions in the human brain. Therefore, we are exploring the role of different connexin subtypes in the lesion of human brain infarction.

Materials and Methods: Brain slice sections were prepared from pathological samples in our hospital. Samples sectioned after brain embolic stroke ($n = 7$) and multiple infarction brains ($n = 4$) were selected for the analysis. We used immunohistochemical analysis to investigate alterations in the expression of connexin subtypes in human stroke brains. The Cx26, Cx32, Cx43 and Cx45 expression was investigated. Data, evaluated semi-quantitatively by computer-assisted densitometry, was compared between the intact hemisphere and ischemic lesions.

Results: Astrocytes were strongly activated in penumbral lesions. The Cx43 expression co-localized with astrocytes was significantly increased in the penumbral area compared to the intact regions. Moreover, the expression of Cx43 was significantly abundant in the lesions of multiple infarctions compared to that of embolic stroke. The co-expression of Cx32 and Cx45 with neuronal markers was significantly increased in the penumbral lesions.

Conclusion: Human brain may respond to ischemic insult by increasing the expression of astrocytic Cx43 for protecting neurons. Cx32, Cx43 and Cx45 may work differently in terms of neuroprotection under brain infarction.

Interactive mechanism of action of REST, microRNA-29c and DNMT3a in post-stroke brain damage

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The transcription factor REST/NRSF prevents the neural specific gene expression in non-neuronal cells. We observed that transient focal ischemia in adult rats induced REST expression in brain and bioinformatics showed several REST binding sties in the miRNA mir-29c gene promoter. Interestingly mir-29c is one of the highly expressed miRNA in normal brain and the most down-regulated miRNA in the ischemic brain. The mir-29c levels were also decreased in PC12 cells subjected to oxygen-glucose deprivation (OGD). Cotransfecting with a REST plasmid prevented mir-29c promoter vector expression in PC12 cells confirming that REST is the upstream transcriptional controller of mir-29c. Furthermore, treating PC12 cells with REST siRNA prevented the post-OGD down-regulation of mir-29c. Bioinformatics indicated that DNMT3a mRNA is a major target of mir-29c and cotransfecting with premir-29c prevented the expression of DNMT3a 3'-UTR vector in PC12 cells. DNMT3a mRNA and protein expression also increased following *in vivo* or *in vitro* ischemia. Recovering mir-29c levels by treating with a premir-29c or inhibiting DNMT3a or REST expression with specific siRNAs prevented OGD-induced cell death by 70 to 80%. On the other hand, treating normal PC12 cells with an antagomir-29c killed $\sim$55% cells within 24h. Furthermore, intracerebroventricular infusion of premir-29c to adult rats decreased the post-ischemic infarction (by $\sim$45%) and neurological deficits compared to a control-mir treatment. Thus, these studies indicate that mir-29c down-regulation after focal ischemia leading to increased expression of its down-stream target DNMT3a might be a putative mediator of ischemic cell death and REST is the upstream transcriptional controller of mir-29c. Funded by NIH grant NS061071 and CURE.

The mitochondrial permeability transition – a broad target for neuroprotection

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In neurodegenerative disease states, sudden increase of the permeability of the inner mitochondrial membrane in response to threshold calcium concentration or oxidative stress leads to the formation of an unselective permeability transition pore (PTP) complex. Intense studies of the PTP phenomenon did not yet allow unraveling the biochemical mystery and the structure of this pore complex. Gene knockout experiments ruled out the earlier accepted involvement of voltage-dependent anion channel and adenine nucleotide translocase as structural elements of PTP. Interestingly, the peripheral benzodiazepine receptor (PBR), now designated the 18-kDa translocator protein (TSPO) of the outer membrane, seems to take part in PTP regulation. We present data on evidence how ligands of TSPO or PBR (PK11195, Ro5-4864, protoporphyrin and diazepam binding inhibitor) are able to modulate the induction of Ca^{2+}-induced PTP in rat brain mitochondria. Furthermore, we summarize the recently revealed contribution of two novel proteins, 2',3'-cyclic nucleotide 3'-phosphodiesterase and $p42^{IP4}$ (centaurin α1; ADAP 1), to Ca^{2+} efflux from rat brain mitochondria loaded by threshold [Ca^{2+}] and thus to induction of PTP. In conclusion, the mitochondria permeability transition pore complex in brain with its interacting proteins presents a promising target for protection in many neurodegenerative diseases

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Prostanoid receptors in ischemic brain injury: Mechanisms and translational potential

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Objective: Prostaglandin E2 (PGE2) type 1 receptors (EP1R) have emerged as an important factor in the mechanism of the cell death associated with hypoxic-ischemic injury. In this presentation we will review the

evidence linking EP1R to ischemic injury in models of focal and global cerebral ischemia. Furthermore, we will examine the participation of EP1R in the dysfunction of cerebrovascular regulation associated with hypertension, a major risk factor for stroke and vascular dementia.

Material and methods: Focal cerebral ischemia was produced by temporary occlusion of the middle cerebral artery (MCA) in mice. Forebrain ischemia was produced by bilateral common carotid artery occlusion. The regulation of the cerebral circulation was investigated in urethane-chloralose anesthetized mice in which cerebral blood flow (CBF) was monitored using a laser-Doppler flow probe placed on the somatosensory cortex through a cranial window. Hypertension was induced by acute (30 min) or chronic (14 days) administration of the pressor agent angiotensin II (AngII).

Results: Administration of the EP1R antagonist SC51089 conferred significance neuroprotection in both focal and forebrain ischemia. The protective effect had a wide therapeutic window (12 hrs), was long lasting (at least 2 weeks) and was observed both in male and female mice. Reduced ischemic injury was also observed in EP1R-null mice following focal or forebrain ischemia. The source of the PGE2 activating EP1R was found to be cyclooxygenase-2 (COX-2). We used neuronal culture to study the mechanisms of the protective effect conferred by EP1R. We found that EP1R enhance the Ca^{2+} dysregulation induced by glutamate excitotoxicity. The effect was attributable to suppression of the activity of the Na^{+}/Ca^{2+} exchanger. EP1R are also involved in the dysregulation of the cerebral circulation induced by hypertension. Administration of AngII increased blood pressure and attenuated the increase in CBF induced by neural activity (whisker stimulation) and endothelium-dependent vasodilators (acetylcholine, Ca^{2+} ionophore, bradykinin). These cerebrovascular effects were not observed in EP1R-null mice or after administration of SC51089 to wild type mice. The source of the PGE2 activating EP1R was found to be COX-1 expressed predominantly in microglia.

Conclusions: These data provide evidence that EP1R are important mediators of brain damage in models of focal and global ischemia. EP1R antagonists are attractive candidates for stroke treatment. Their wide therapeutic window, the sustained nature of the protection, their effectiveness in both sexes, and their efficacy in focal and global ischemia are highly desirable features with great translational potential. The efficacy of EP1R antagonist in preventing the deleterious cerebrovascular effects of AngII implicates EP1R in the mechanisms of the cerebrovascular complications of hypertension. Therefore, EP1R antagonists could have a broad use in the prevention and treatment of cerebrovascular diseases.

Growth factors

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Pharmacological agents, such as neural growth factors, seem to be attractive tools to facilitate protection and recovery in traumatic and neurodegenerative disease. Recently uncovered direct CNS action of factors recently known to control differentiation of white and red blood cells uncovered hematopoietic factors (Granulocyte-colony-stimulating factor, Erythropoietin) as promising candidates. Numerous data from cell culture studies and animal models in stroke, ALS, and Parkinsons's disease have shown that those factors pass the blood-brain barrier, acts on neurons and counteract cell death. A number of mechanisms of action in the CNS have been identified, the most relevant relating to neuroplasticity, stem cell proliferation and differentiation and may enhance recovery in stroke and neurodegenerative diseases, even when treatment with these factors starts at delayed time intervals. Additionally, interesting for a further clinical evaluation of these factors is their long clinical history in other indications with an excellent safety record.

Inhalation of nitric oxide prevents ischemic brain damage

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Stroke is the 3rd most common cause of death in industrialized countries. The main therapeutic target is the ischemic penumbra, potentially salvageable brain tissue which dies within the first few hours after blood flow cessation. Hence, strategies to keep the penumbra alive until reperfusion occurs are needed. The presented experimental study demonstrates that inhaled nitric oxide (iNO) leads to the formation of NO-carriers in blood which distribute throughout the body. While under normal conditions iNO does not affect cerebral

blood flow, following experimental cerebral ischemia it selectively dilates arterioles in the ischemic penumbra, thereby increasing collateral blood flow and, hence, significantly reducing ischemic brain damage. iNO may thus provide a completely novel strategy to improve penumbral blood flow and neuronal survival in stroke or other ischemic conditions where collateral blood flow is present.

Reduction of cerebral infarct size by dronedarone

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Background: In the ATHENA trial, dronedarone reduced the incidence of stroke in patients with atrial fibrillation. Since smaller cerebral infarcts are sometimes asymptomatic, the reduced incidence of stroke might reflect a reduction of infarct size (IS) by dronedarone. However, no data on the effect of dronedarone on cerebral ischemia/reperfusion injury are available.

Methods and results: In 60 rats, the middle cerebral artery was occluded (MCAO) with a suture for 1h followed by reperfusion. IS was assessed at day 7 by TTC-staining. Animals were examined using a neurological 5 points score. Twelve animals served as controls (group A), 12 animals received 30 mg/kg (group B) and 100 mg/kg (group C) dronedarone daily starting 3 days before MCAO; 12 animals received 30 mg/kg (group D) starting 2 h after MCAO. In all groups treatment was maintained until day 7. In 12 additional animals (6 controls, 6 animals pretreated with 30 mg/kg) fractional anisotropy (FA) was assessed using magnetic resonance imaging (MRI). IS in group A was $151 \pm 45\,mm^3$ versus $94 \pm 42\,mm^3$ in group B, $79 \pm 29\,mm^3$ in group C, and $127 \pm 51\,mm^3$ in group D, respectively (B,C,D $P < 0.05$ vs. A). Neuroscores and weight loss (expressed as percent of initial weight) were less in treatment groups: 1.8 ± 0.6 and 91% in group B, 1.4 ± 0.5 and 93% in group C, and 2.1 ± 0.6 and 89% in group D compared to 2.4 ± 0.5 and 83% in controls (B,C,D $P < 0.05$ vs. A). FA in the ischemic penumbra was significantly higher in treated than in control animals (0.44 ± 0.2 vs. 0.35 ± 0.17; $P < 0.05$).

Conclusions: Dronedarone administered before and after MCAO reduces IS and improves FA and neurological outcome in transient cerebral ischemia.

VCAM-1 is a key molecule in cerebral leukocyte invasion after experimental brain ischemia

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Objectives: Cerebral invasion of systemic immune cells represents a crucial step in post-ischemic neuroinflammation. Vascular Cell Adhesion molecule-1 (VCAM-1) is of prime importance for endothelial adhesion and transmigration by mediating the interaction between leukocyte integrins with the activated endothelium. Herein, we compare for the first time the blockade of cerebral VCAM-1 by specific antibodies or gene silencing of VCAM-1 by *in vivo* siRNA administration.

Materials and methods: Permanent focal cerebral ischemia was induced by transtemporal middle cerebral artery occlusion (MCAO). VCAM-1 expression was analyzed by western blot (WB) and immunohistology. Mice were treated with VCAM-1-specific antibodies at 24 h prior and 3d after MCAO, controls received IgG2 isotype. The specific and effective antibody-binding was controled by immunohistology and fluorescence spectrometry. In a second experiment, mice received VCAM-1-specific siRNA or control siRNA by intravenous hydrodynamic injection. Infarct outcome was investigated by infarctvolumetry, leukocyte invasion was analyzed by immunohistology, systemic and cerebral cytokine expression was measured by ELISA, RT-PCR and WB.

Results: VCAM-1 is specifically upregulated after MCAO in the periinfarct area and its expression correlates with the kinetic and local distribution of invading immune cells. High doses of Anti-VCAM-1 antibodies effectively block endothelially expressed VCAM-1. However, VCAM-1 blocking by antibodies did not improve infarct outcome nor did it significantly reduce the cerebral invasion of leukocytes or the expression of proinflammatory cytokines after MCAO. In contrast, treatment with VCAM-1-specific siRNA significantly reduced cerebral VCAM-1 expression by 75% on RNA- and by 30% on protein-level and particularly inhibited the prolongated upregulation of VCAM-1 after MCAO. At 6d after MCAO, the invasion of granulocytes and T cells in the treatment group was reduced by $> 80\%$ compared to controls. Likewise, VCAM-1 siRNA administration resulted in a massive reduction

of cerebral IFN-γ expression by $>$ 90% compared to controls.

Conclusions: VCAM-1 gene silencing, but not the direct inhibition by blocking antibodies, effectively reduces the cerebral leukocyte invasion after MCAO and its related induction of inflammatory cascades. This new approach provides a potent therapeutic option for more specific interventions in post-ischemic neuroinflammation.

Targeting the deficiency in alpha-linolenic, the omega-3 polyunsaturated fatty acid precursor, of the western diet: A strategy for stroke prevention and treatment

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Objectives: Omega-3 intake insufficiency, both in the form of alpha-linolenic acid (ALA) and its long chain derivatives, is suspected to be a risk factor for stroke in Western countries populations. Stroke is a major cause of mortality and morbidity and induces significant socioeconomic cost and a marked increase in patient/family burden. To date, preventive treatments and neuroprotective drugs identified in preclinical studies failed in clinical trials. Because of this poor track record for stroke drug development in clinic, dietary functional foods/nutraceuticals may constitute an important tool in preventing stroke damages. While several epidemiologic studies suggested a beneficial effect of a seafood-enriched diet in cerebral diseases, very few works have investigated vegetable oils rich in ALA as a brain protectant against stroke. Therefore, our work aimed to design and compare preventive strategies by ALA supplementation either by injections or by experimental diets on cerebral ischemia.

Results: This talk will show that ALA-mediated protection targets the neurovascular unit acting both on neuronal and vascular cells as well as time-dependent mechanisms and that ALA is a pleiotropic agent.

A focus would be done on five particular benefits of ALA:

1) ALA acts on neurons. ALA injection reduces acute ischemic damages limiting glutamate-mediated excitotoxicity and thus neuronal death both *in vivo* and *in vitro*.
2) ALA has vasoactive properties. it vasodilates brain collateral arteries *ex-vivo* that account for the improvement in cerebral blood flow observed after an ALA injection *in vivo*.
3) Repeated injections of ALA as pre- or post-treatment protect from stroke.
4) *In vivo*, ALA treatment *per se* promotes neuronal plasticity (neurogenesis and synaptogenesis), neurotrophic factors expression and antidepressant activity. Stimulating each of these factors is known to promote stroke recovery. *In vitro*, ALA application on mature neurons or neural stem cells also induced neuronal plasticity and neurotrophic factors expression, suggesting a direct effect of ALA and not of its long chain metabolites.
5) Diets containing rapeseed oil as only source of lipids, rich in ALA prevent the damage induced by middle carotid artery occlusion. Significant reduced mortality rate, infarct size and increased probability of spontaneous reperfusion in the post-ischemic period were also observed with diet enriched in ALA.

Conclusion: ALA by $i.v.$ treatment could protect from stroke and boost functional stroke recovery by potentially combining acute neuroprotection with long-term repair/compensatory plasticity. ALA supplementation through a slight modification of the daily diet employing rapeseed oil as nutraceutical could help in stroke prevention. Finally, the multi-potential effects of ALA may be used to create a global strategy for protecting and stimulating brain responses to achieve post-ischemic functional recovery.

Intravenous coadministration of smooth muscle and endothelial progenitor cells enhances neurogenesis and angiogenesis, and reduces behavioural deficit in cerebral ischemia in mice

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Extensive evidences indicate that angiogenesis plays a key role in stroke recovery. Therefore, we assessed

whether coadministration of smooth muscle progenitor cells (SMPCs) and endothelial progenitor cells (EPCs) isolated from human cord blood enhances angiogenesis, neurogenesis and neurological recovery in two mouse models of focal cerebral ischemia.

Methods: Ischemia was induced in adult male C57Bl6 mice (1) permanently by thermocoagulation of the middle cerebral artery (MCA) or (2) transiently by 45 min intravascular occlusion of the MCA. SMPCs (0.5×10^6), EPCs (0.5×10^6), SMPCs+EPCs ($0.25 \times 10^6 + 0.25 \times 10^6$) or PBS were intravenously administered 24 hours post-MCA occlusion ($n = 6$–8/group). Infarct volume, blood-brain barrier (BBB) permeability, vascular area, cell proliferation and proliferating cell phenotype were assessed at days 7 and 14 after permanent ischemia. Neurological deficit was evaluated 3, 7 and 14 days after transient ischemia.

Results: Seven days after permanent ischemia, vascular area in the infarct was significantly increased in all treated groups with SMPCs, EPCs or EPCs+SMPCs, compared to controls ($+17.4\% \pm 8\%$, $+19.4\% \pm 5.2\%$ and $+17.3\% \pm 3.6\%$, respectively, $p < 0.05$) and remained increased in the co-treated group at day 14 compared to the PBS ($+11.7 \pm 1.5\%$, $p < 0.05$). However, SMPCs and EPCs-treated mice showed an immature disorganized vasculature, whereas co-treated mice showed a mature network from day 7. The number of co-labelled BrdU-CD31 positive cells was significantly increased in all treated groups: 14.7 ± 1.2/ROI (PBS), 22.7 ± 1.5/ROI (SMPCs), 33.3 ± 3.2/ROI (EPCs) and 35.0 ± 1.4/ROI (SMPCs+EPCs) ($p < 0.01$) at day 7. The number of peri-infarct BrdU-positive cells was higher only in the co-treated group (3-fold) compared to the three other groups ($p < 0.01$) at day 7, that appeared to be in part GFAP/BrdU cells (30 ± 7 vs. 13 ± 2/ROI, $p < 0.005$). In this group, significant neurogenesis and migration of neuroblasts towards the perischemic area were observed at day 14 (19 ± 4 vs. 13 ± 2/ROI in the control group, $p < 0.05$). Infarct volumes and BBB permeability were not significantly modified at day 7 and day 14. Three days after transient ischemia, co-treatment improved neurological recovery evaluated by grip + string tests ($p < 0.05$).

Conclusion: To conclude, co-administration of human cord blood SMPCs and EPCs triggers early vascular remodelling, angiogenesis in the infarct area, recovery of neurological deficit and maintenance of neurogenesis at day 14 after cerebral ischemia.

Human umbilical cord-blood mesenchymal stem cells induce long term protection after brain trauma in mice

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Question: human umbilical cord blood mesenchymal stem cells (CB-MSC) have been recently identified as an easily available, novel source of progenitors with multilineage potential. Here we investigated if CB-MSC: 1) decrease traumatic brain injury sequelae and restore brain function; 2) are able to survive and migrate in the injured brain; 3) induce relevant changes in the environment in which they are infused.

Methods: CB-MSC were isolated from cord blood through a negative depletion of the erythroid component. C57Bl/6 mice were subjected to controlled cortical impact (CCI)/sham brain injury. At 24h postinjury they received an intracerebroventricular injection of CB-MSC (150,000/5ul) or PBS (control group) contralateral to the injured side. Immunosuppression was achieved by cyclosporine A (10 mg/kg ip). Neurological motor function was evaluated by Neuroscore (NS) and Beam-Walk (BW) tasks. Cognitive function was assessed by Morris Water Maze (MWM). The expression of brain derived neurotrophic factor (BDNF) was determined by western blot analysis. The activation of microglia/macrophage and the effect on gliotic scar was determined by immunohistochemistry. For BW, NS, MWM, contusion volumes and BDNF concentrations, the comparison between groups was performed by 2-way ANOVA and Tukey post hoc test. Unpaired t-test was used for CD11b, CD68 and GFAP quantification.

Results: CB-MSC transplantation significantly improved NS and BW performance at 2, 3 and 4 weeks postinjury compared to controls; [4 weeks postinjury, median NS: 7 (range: 6–9) and 5 (3–9); BW: 24 (8–37) and 38 (24–51) in CB-MSC transplanted and control mice respectively, $n = 16$]. Moreover 1 month postinjury CB-MSC mice also showed attenuated learning dysfunction at MWM ($p < 0.05$) and reduced contu-

sion volume compared to control mice (13.7 ± 1.7 mm^3 and 17.3 ± 1.3 mm^3 respectively, $p < 0.01$). Assessment of cell distribution revealed that Hoechst positive CB-MSC survived in the injured brain up to 5 weeks and homed to lesioned tissue as early as 1 week after injury in 67% of mice. By 3 days postinjury, cell infusion significantly increased BDNF concentration in cortical contusion core and bordering region restoring BDNF expression close to the levels observed in sham operated mice ($n = 8$). By 7 days postinjury, we observed a selective rise (260%) in CD11b-positive cells of CCI CB-MSC compared to control mice. Conversely we observed a significant decrease (58%) in CD68 (a marker of active phagocytosis) positive cells in CCI CB-MSC compared to control mice indicating a non phagocytic activation of microglia/macrophages. Thirty-five days after injury, in the scar region, CCI CB-MSC mice showed a selective decrease of GFAP positive cells compared to control mice ($n = 8$, $p < 0.01$).

Conclusions: these findings suggest that CB-MSC stimulate the injured brain and evoke protective events through trophic and immunomodulatory mechanisms that remodel the brain and lead to significant improvement of neurological outcome.

Animal models in ischemic research – Why has translational research to humans been so elusive?

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Brain protection from focal or global cerebral ischemia is not new, not innovative, and so far, mostly not successful. There have been many methods and agents tried in an attempt to produce neuroprotection from cerebral ischemia: hypothermia, anesthetics, free radical scavengers, excitatory amino acid antagonists, calcium channel blockers, ionic pump modulators, growth factors, heparinization, anti-neutrophil/platelet factors, sex steroids, and genes and gene products. There are hundreds, perhaps thousands of neuroprotective drugs that have been used in animal models. So, if you were a mouse or a rat, and suffered a stroke or cardiac arrest, we would know just what to do for you. But, essentially none of these pharmacological agents have demonstrated usefulness in humans even though they have been shown to be successful in pre-clinical animal trials. What could account for this and why do we not have a magic bullet?

One issue is that there are several important mechanisms of injury from focal and global ischemia, and the agents mentioned above usually work only on one potential mechanism. For example, ischemia can produce free radicals, result in the release of excitotoxins, and release mediators of inflammation, and there are other mechanisms of injury as well. All of these mechanisms of injury can result in neuronal cell death. Since there are multiple mechanisms of injury, it is likely that there are multiple mechanisms of neuroprotection. None of the agents mentioned above can affect all mechanisms of injury at once or at the appropriate time following injury. Another issue is that there is a temporal sequence of injury following ischemia. For example excitotoxity occurs very quickly, in minutes or hours after the ischemia. Inflammatory injury or apoptotic injury may not occur until hours or even days after the injury. There is even a temporal sequence of gene expression such that heat shock proteins and early genes are expressed early after the ischemia, whereas growth factor genes are expressed days after injury. Thus the pharmacologic agents must be administered at the appropriate time to result in neuroprotection.

Finally, there are other issues concerning drug dose and time of administration of the drug which can determine neuroprotection efficacy. A dose of drug that is effective in a mouse may not be the correct effective dose in a human. The window of opportunity in a mouse also may not be the correct window in the human. Combination therapy also needs to be attempted in humans. In addition, in humans there may be a genetic predisposition for injury from ischemia, and gender differences. Issues related to clinical trial design and analysis also must be considered. We must also consider that we work mostly with normal, young, healthy animals, whereas in the human population this is not true. Finally, we must also ask the question of whether the animal models of ischemia we use, accurately reflect ischemic disease in humans.

Delayed citalopram treatment affords neuroprotection and behavioral recovery in a mouse model of poststroke depression

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Although poststroke depression is a frequent chronic neuro-psychiatric complication of stroke with great relevance for functional recovery and long-term survival,

the underlying pathomechanisms remain poorly understood. Here, we examined the behavioral phenotype of mice at 14 weeks after 30 min middle cerebral artery occlusion (MCAo)/reperfusion or sham operation. Following left, but not right, MCAo, animals developed an 'affective' phenotype characterized by anhedonia, increased anxiety and despair. This depression-like syndrome was associated with structural and functional alterations of the mesolimbic reward system after stroke. MCAo resulted in delayed exofocal neurodegeneration of dopaminergic neurons in ipsilateral midbrain, which was accompanied by reduced dopamine concentrations and decreased levels of dopamine transporter density along with increased brain-derived neurotrophic factor (BDNF) protein levels in ischemic striatum. Chronic daily treatment with the selective serotonin reuptake inhibitor (SSRI) citalopram, which was initiated 7 days post event, completely reversed the depressive phenotype in MCAo mice. Importantly, citalopram treatment also largely prevented degeneration of dopaminergic midbrain neurons and attenuated the extent of striatal atrophy at four months. Reduction of secondary neuronal injury may provide a novel target to afford recovery after brain ischemia and underlie the clinical benefits of antidepressant therapy after stroke.

Towards making drug treatment the rule instead of an exception: Antibody-based therapy for Stroke

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Tissue-type plasminogen activator (tPA, endogenous and exogenous) has two faces in acute ischemic stroke: beneficial fibrinolysis in the vascular bed, but damaging effects on the neurovascular unit and the brain parenchyma. To improve this profile, we used our knowledge of the underlying molecular (patho) physiology to develop a novel treatment strategy for stroke, relying on antibodies targeting the pro-neurotoxic effects of tPA. Based on a dedicated model of murine thrombo-embolic stroke, we demonstrate the efficiency of immunotherapy in a complete pre-clinical screen: after a single administration (alone or with late thrombolysis), antibodies dramatically reduce ischemic brain injuries, improve long-term neurological outcome and, in parallel, attenuate critical ischemic events including blood-brain barrier leakage and activation of MMP-3, MMP-9, and the PDGF-CC pathway. Thus, the prospect of this immunotherapy is an extension of the range of treatable patients, whether used as a monotherapy or, in combinations, to extend the therapeutic window for thrombolysis.

Cross-talk and developmental programs in stem cell biology – A key to translational neuroscience

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We have most recently been studying, an intriguing phenomena, (with possible therapeutic implications) that has begun to emerge from our observations on the behavior of neural stem cell (NSC) clones in various mouse and primate models of CNS injury and degeneration. During phases of active neurodegeneration, factors seem to be transiently elaborated to which NSCs may respond by migrating (even long distances) to degenerating regions where they attempt to restore homeostasis by a variety of mechanisms. This may include, (but is not limited to) differentiating, towards the replacement of degenerating neural cells of multiple types, not only neurons but also requisite non-neuronal "chaperone" cells, all of which are essential for the proper development and reconstitution of function. NSC's are drawn to inflammatory niches, where they then exert anti-inflammatory actions. These repair mechanism may also reflect the re-expression of basic developmental programs (particularly during temporal windows following injury). There is an enormous amount of "programmed" cross-talk between stem cells and the milieu that add complexity but also enrich therapeutic promise to the system. In addition, NSCs in their native state (as well as following genetic-engineering) may serve as vehicles for protein delivery allowing for the possibility of simultaneous cell replacement and gene therapy (e.g., with factors that might enhance differentiation, neurite outgrowth, connectivity, neuroprotection, anti-inflammation, anti-scarring, and angiogenesis). Cell-cell contact with communication through gap junctions appears to represent another mode of cross-talk. Multi-model approaches to most neurological conditions are likely required. The stem cell may serve as the "glue" for these. When combined with certain synthetic biomaterials, NSCs may be even more effective in engineering the damaged CNS towards reconstitution. Not only gene expression programs, but also an epigenetic chromatin modification programs seem critical for dictating plas-

ticity and potency. These chromatin structures appear to influence the expression of various stemness genes, including some novel zinc finger proteins that influence the unfolding of various developmental programs along the continuum from pluripotence (as ES cells) to multipotence (as somatic stem cells, e.g., NSCs) to cell type commitment (e.g., as neural cell types).