Poster Session 3: Advanced Therapeutics

P I - 3-1

Stimulation of axon growth in the *in vitro* model of CNS injury by transgenic activation of alpha9 integrin combined with biomodified material

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Regeneration of injured axons in the adult mammalian central nervous system (CNS) is very limited. One of the reasons for poor regeneration is because CNS axons lack an integrin receptor that interacts with tenascin-C, the extracellular matrix glycoprotein of the CNS which is upregulated after the injury. In this project, we investigated whether the regeneration ability of axons could be enhanced by specific integrin activation in combination with a biomaterial modified with appropriate integrin ligands. We have focused on alpha9 integrin subunit (alpha9) specific for tenascin-C and an integrin activator kindlin-1.

As an *in vitro* model of axon growth, we used cultured sensory neurons isolated from dorsal root ganglions (DRG) of adult rats. The DRG neurons were transfected with plasmids for alpha9 or kindlin-1. In the presence of aggrecan, simulating an inhibitory environment after CNS injury, an overexpression of kindlin-1 enables the adult DRG neurons to project long neurites overcoming the aggrecan inhibition. To verify the effect of the alpha9 integrin on axon growth, we used alpha9 specific adhesion peptide derived from tenascin-C (AEIDGIEL) coated on the cover glass or immobilized into fibrin gels.

We suggest that transgenic activation of alpha9 integrin subunit can promote *in vitro* axon growth on the substrate modified with an appropriate peptide ligand and thus overcome inhibitory environment. On the basis of these results, the subsequent *in vivo* study in the model of spinal cord injury using an integrin viral transduction and biomaterial implantation will be performed.

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P I - 3-2

Evaluating the brain biocompatibility of new self-assembling peptide hydrogels for therapeutic use in brain tissue engineering and regeneration after stroke

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Stroke is the leading cause of adult disability worldwide. Current treatments are focused on limiting the damage, and no treatments that target brain regeneration to promote functional recovery exist. After a stroke, limited endogenous neuroregeneration takes place, which can be triggered using stem cell therapy. Indeed, post-stroke angiogenesis and neurogenesis are known to occur in experimental stroke models in response to stem cell administration via direct cell replacement or paracrine effects. However, the fluid-filled stroke cavity resulting after a stroke does not structurally support neural repair. Moreover, stem cell-derived pro-regenerative and trophic factors have a short half-life. Soft biomaterials such as hydrogels may address these two issues, through a number of mechanisms including providing support for regeneration, acting as a scaffold for the survival of implanted cells or acting as a sustained release system of therapeutic biomolecules produced by stem cells. Self-

assembling peptide hydrogels are of particular interest for brain tissue engineering and regeneration. These are characterised by controlled synthesis, biodegradability, non-harmful by-products useful in cell metabolism and tuneable stiffness. For their future use in stroke therapy, peptide monomer immunogenicity and neurotoxicity need to be evaluated, and the aim of this study was to investigate the biocompatibility of self-assembling peptide hydrogels with brain tissue, by examining the effect of hydrogel degradation products on mouse brain cells in primary cultures. Mixed glial or endothelial cells cultures were treated with increasing concentrations of hydrogel monomer solutions and/or with supernatant obtained after hydrogel incubation in culture media. Cell viability, cytotoxicity and release of soluble interleukin-6 (IL-6) and mouse tumour necrosis factor alpha (TNF-α) were assessed. The presence of hydrogel monomers or degradation products did not appear to activate primary microglial cells or astrocytes, as only extremely low levels of the inflammatory cytokines IL-6 and TNF-α were detected. Furthermore, no cytotoxic effects were observed. Finally, self-assembling peptide hydrogels failed to induce expression of cell adhesion molecules in endothelial cells. These results provide evidence that this novel self-assembling peptide hydrogel does not induce an immunogenic response in mouse brain cells, suggesting that it might be a successful biomaterial candidate for brain tissue engineering and regeneration after stroke.

P I - 3-3

Stem Cell-Drug Interactions: An Important, but Neglected Aspect of Future Neuronal Stem Cell Therapy. A Systematic Review and Meta-analysis

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Introduction: Stem cell therapy is a promising treatment option for neurodegenerative diseases (NDD). NDD mostly affect the elderly who often suffer from comorbidities requiring multiple medications. While the interaction between different drug classes is well investigated, almost nothing is known regarding the interaction between stem cells and drugs. Here, we focus on potential interactions between drugs used to treat NDD comorbidities or sequelae and stem cells which may influence treatment efficacy.

Question: To determine the effect of commonly used drug in the elderly on neuronal stem cell.

Methods: We systematically searched PUBMED to identify articles published between January 1, 1991 and November 28, 2016. In vitro and in vivo studies as well clinical trials were included. Only studies investigating effects on mammalian cells were included.

Results: From 5304 publications initially identified, 202 studies were finally included in the systematic review. Only 39 studies provided complete datasets (number of sample, mean, and standard deviation) as required for meta-analysis, and these studies varied across drug classes and effect on proliferation as well as differentiation. We found that antidepressants stimulated neuronal stem cell proliferation (Hedges" g SMD, 0.83; 95% CI, 0.32 to 1.34; p=0.001). Similarly, the most commonly studied antidepressant subclass, selective serotonin reuptake inhibitors (SSRIs), significantly induced proliferation of neuronal stem cells (Hedges" g SMD, 0.83; 95% CI; 0.20 to 1.46; p=0.010). We also identified several other potential interactions (such as interaction of drugs with stem cells in the ischemic/hypoxia model) but the limited number of available datasets precludes robust conclusions.

Conclusions: Despite the fact that available data were hardly sufficient to perform meta-analysis in most cases, a clear interaction between SSRIs and neuronal stem cells was identified. Moreover, we found preliminary evidence for potential further interactions. This clearly warrants a more detailed investigation. We further recommend to assess how pharmacological interventions and stem cells can be combined in more efficient, safe, and ultimately successful therapeutic strategies.

P I - 3-4

Antioxidant properties of innovative polymer coated cerium oxide nanoparticles for stroke treatment

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Questions: The deleterious role of oxidative stress in neuronal but also vascular lesions following ischemic stroke is clearly established. However, so far no antioxidant strategy has shown clinical efficacy. Cerium oxide nanoparticles (CNPs) have interesting antioxidant capacities via scavenging free radicals and mimicking antioxidant enzymes activities. The objective of our project is to develop innovative polyethylene glycol/phosphonate copolymers coated CNPs to improve their stability and prolong their circulation time in order to prevent endothelial oxidative stress and subsequent vascular lesions and hemorrhagic transformations. The first part of the project presented here evaluates the potential toxicity, the antioxidant capacities and the internalisation of these new CNPs *in vitro*.

Methods: Studies were conducted on bEnd.3 cells (a murine endothelial cerebral cell line) treated with glutamate to mimic ischemia-induced oxidative stress *via* excitotoxicity. Three different coated CNPs and bare CNP were incubated at 10, 100, 1000 µg/mL for 4h and 24h. Toxicity was evaluated by quantification of the metabolic activity (MTT) and mortality (Trypan Blue) of the cells. Reactive oxygen species (ROS) production was detected with the fluorescent H_2DCF-DA probe. Cyanine-labelled CNPs were used to study the nanoparticles internalisation/localisation by FACS and fluorescence microscopy. Subcellular localisation was determined by transmission electron microscopy.

Results: A decrease in metabolic activity was only observed with CNPs at the highest concentration. Bare CNPs induced cell death but only at the highest concentration (1000 µg/mL): 29% at 4h and 36% at 24h. Cell death remained below 5% for all other CNPs whatever the concentration. With regard to oxidative stress, glutamate increased ROS production by 41% at 4h and 46% and 24h. All CNPs exhibited an antioxidant effect at 100 and 1000 µg/mL. Cyanine labelling of the CNPs allowed quantifying the interaction of CNPs with bEnd.3 cells at 4h (86%) and 24h (98%). These data were confirmed by images acquired through fluorescence microscopy and CNPs internalisation was observed at the cytoplasmic level, especially in the perinuclear region. Electron micrographs showed the CNPs to be located in the endosomes.

Conclusions: The coating of CNPs decreases their toxicity while maintaining their antioxidant properties and allowing their internalisation. CNPs toxicity and bioavailability will now be assessed *in vivo* in a mouse model of cerebral ischemia to further evaluate their therapeutic potential.

P I - 3-5

Development of 3D cellular microenvironments as potential therapy for brain repair after ischemic stoke

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Disruption of cerebral blood flow in the brain during ischemic stroke results in loss of nervous tissue causing severe disabilities or death. Despite the major socioeconomic impact of stroke on health services, the limited available treatments create the need for the development of new therapeutic interventions for brain regeneration. Transplantation of stem cells at the infarction site aims to repair the damaged brain by replenishing the lost tissue. Currently, clinical translation of stem cell-based interventions is prevented by poor cell survival and functional integration into the host tissue. Extracellular matrix-based hydrogels can be used to promote cell integration into the host by providing spatial and mechanical support for cell adhesion and survival. Assembly of the neuronal and vascular cell populations into functional neurovascular units infers that communication between cell types is required for brain homeostasis. Based on the bidirectional communication between neurovascular cells, it is hypothesized that a hydrogel scaffold composed of natural biopolymers can support functional integration of induced pluripotent stem cell-derived neural stem cells and endothelial cells to re-establish neural networks and promote the reconstruction of the local vasculature respectively. For this, it is of interest to examine whether combinations of vascular and neural cells in a 3D hydrogel can be used to *in vitro* model neuronal tissue. Here, focusing on the derivation of the neuronal and vascular cells, it is shown that human induced pluripotent stem cells can be successfully differentiated for the derivation of neuronal progenitor cells that display characteristic neuronal progenitor morphology and function. Additionally, induced pluripotent stem cell differentiation towards an endothelial phenotype results in populations of functional brain-specific endothelial cells. Combination of neural progenitor and endothelial cells can be used for the establishment of 3D neurovascular networks for the development of a hydrogel-based cell transplantation therapy for brain repair.

P I - 3-6

Optimising the biodistribution of mesenchymal stem cells in a preclinical mouse model of ischaemic stroke to enhance the therapeutic potential of primed mesenchymal stem cells for therapy

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Backgorund and aims: Stroke is a leading cause of death and disability worldwide but with limited therapies, thus new therapeutic strategies are urgently needed. Inflammation after stroke is associated with poor outcome and is thus an attractive therapeutic target. A key mediator of inflammation is the cytokine interleukin-1 (IL-1), and blocking IL-1 actions have shown promise for acute neuroprotection and recovery in preclinical stroke models, and is currently tested in stroke patients. A potential new approach in stroke therapy is the targeted application of human mesenchymal (stromal) stem cells (MSCs) that can exert potent anti-inflammatory, neuroprotective and regenerative actions. MSCs can be primed by preconditioning with IL-1a treatment to exert potent anti-inflammatory, neuroprotective and regenerative actions in clinically-relevant animal stroke models for their potential use in future stroke therapies. The use of primed MSCs has not been previously tested in stroke, therefore it is essential to characterise the biodistribution of these cells to maximise their therapeutic potential by optimising cell labelling and tracking protocols.

Methods: MSCs were labelled with the near infra-red cell dye DiD and co-dyed with Cell Tracker, prior to injection, via intra-arterial or intravenous route, after experimental focal cerebral ischaemia. Animals were saline

and paraformaldehyde-perfused 24 h post-stroke and organs were excised for imaging and histology. DiD dye labelling enabled the use of the *In Vivo* Imaging System (IVIS) to indirectly quantify the biodistribution of labelled MSCs and the co-dyeing with CellTracker allowed better visualisation of MSCs in the brain and peripheral organs for histology.

Results: MSCs are more likely to reach the brain, particularly the area of stroke damage, after intra-arterial administration compared to intravenous injection and both dosing methods resulted in MSCs to be located in the lungs, liver and spleen. This effect varied between experiments for intravenous administration with low cell numbers suggesting a high rate of peripheral clearing. Lesion volume was decreased in mice that received MSCs intra-arterially but not intra-venously adding to evidence on safe doses for intra-arterial injection of MSCs.

Conclusion: This study suggests that intra-arterial dosing is more efficient than intravenous administration of MSCs, due to greater central and peripheral distribution of cells. Co-labelling MSCs with DiD and Cell Tracker dye is another useful tool that allows us to better understand the biodistribution of MSCs via different dosing methods and can potentially enhance the therapeutic potential of using primed MSCs for stroke therapy.

P I - 3-7
Microfilament-engineered midbrain organoids as a new model to study midbrain-associated neurodegenerative diseases
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Recent progress in 3D cell culture systems enables generation of midbrain organoids derived from human induced pluripotent stem cells (iPS). However, organoids architecture and differentiation capacity are still variable between cultures. Moreover, prolonged cultures often lead to necrosis inside growing organoid.

Therefore, the main goal of this study was to overcome these serious problems in the production of organoids using carbon and poly (lactide-co-glycolide) microfilaments.

We compared the influence of these scaffolds on organoid growth and differentiation. We found that carbon fibers accelerate the growth of midbrain organoids and changes their architecture in comparison to PLGA. Furthermore, carbon microfilament-engineered midbrain organoids display similar structure as observed in human substantia nigra pars compacta. Thus, this organoids model may provide a reproducible *in vitro* system to study the human midbrain and its related diseases.

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P I - 3-8

Large-scale generation of transplantable induced motor neurons (iMN) from human fibroblasts facilitates functional recovery after spinal cord injury by cell therapy

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The production of patient-specific motor neurons offers clinical opportunities to cure spinal cord injury patient. Induced pluripotent stem cells (iPSCs) hold great promise for cell therapy. However, their low efficiency of differentiation and tumorigenesis severely hinder clinical applications. The development of cell reprogramming has provided an alternative and effective method for generating patient-specific cells avoiding the tumorigenesis inherent with iPSCs. Nevertheless, previous direct lineage conversion protocols remain the challenges for the therapeutic purpose due to limited number of converted cells. Here, we demonstrate that human fibroblasts can be directly and efficiently converted into highly pure induced motor neurons (iMNs) by sequential transduction of defined two transcription factors. Sequential introduction of transcription factors enables large-scale production of highly pure iMNs through proliferative intermediate state. These iMNs exhibit cytological features of spinal motor neurons, including cellular morphology, gene expression signature, electrophysiological properties, synaptic activity and the formation of neuromuscular junctions (NMJs). Notably, transplanted iMN display engraftment capacity and contribute to recovery of hind limb locomotor function in rodent spinal cord injury model *in vivo*. In conclusion, we established a new reprogramming strategy to acquire a large number of iMNs for cell replacement therapy after spinal cord injury. This proof-of-principal study may offer a novel, safe and effective approach to generate patient-specific cells and open new insights into future regenerative medicine for neurological disorders.

P II - 3-9

Nanoparticle-mediated Blood-Brain Barrier Transport for Neuronal Delivery in Focal Brain Ischemia

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Introduction: Dendrimers are special three-dimension macromolecules with a nano-scale dimension and a highly branched structure, which have a tremendous potential as delivery systems. In central nervous system pathologies, including brain ischemia. Dendrimers have therefore been applied with therapeutic purposes, being PAMAM the most extensively described.

Materials and Methods: Poly(amido amine) (PAMAM) dendrimers of different generations were functionalized with poly(ethylene glycol) (PEG) to reduce cytotoxicity and prolong blood circulation half-life, aiming for a safe in vivo drug delivery system in a stroke scenario. Rhodamine B was used as a small surrogate drug as well as for tracking purposes. We have selected PEGylated G4 PAMAM dendrimers functionalized with Rhodamine (PEG-PAMAM-Rhod) for analysis in an in vitro blood-brain-barrier-model and in a mice model of permanent brain ischemia. The fate of the dendrimers was investigated, focusing on the brain and some peripheral organs, by following Rhodamine after intravenous administration post-injury. Moreover, hemolysis and clotting assays were performed to assure biocompatibility after intravenous administrations.

Results: The presence of PEG-PAMAM-Rhod dendrimers was significantly increased in the brain cortex after ischemia when compared to unlesioned tissue. Moreover, this increase was not observed upon free Rhodamine administration. The dendrimers could be detected inside neurons, including those in the ischemic area. The inspection of the choroid plexus of the brain showed a high accumulation of dendrimers in unleasioned animals while it was decreased when administered in the post-lesion period. Nonetheless, similar to what was observed in the brain cortex, the presence of free Rhodamine is minor when compared to PEG-PAMAM-Rhod dendrimers. Besides the brain, these dendrimers were also detected in the liver and kidney as a result of the selected route of administration. Moreover, the contact of the PEGylated dendrimers with red blood cells was safe as no hemolysis was detected contrary to the unPEGylated parent dendrimer. Additionally, the PEGylated dendrimers behaved as anti-coagulation factor since the clotting period was substantially delayed.

Conclusions: Upon injury such as in ischemic stroke, the blood-brain barrier is compromised which allows for the entrance of molecules that otherwise would be excluded and also for the increase of the flux and entry of substances and cells. Our data reflects this response with a much higher presence of PEGylated dendrimers bound to Rhodamine in the ischemic brain, 24 hours after administration. Such a result points to the dendrimers being an effective delivery vehicle for therapeutic drugs towards the injured central nervous system.

P II - 3-10

The effect of erythropoietin receptor activation with carbamylated darbepoetin (CdEPO) in the brain cells on cognitive functions of C57BL/6 mice

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At present, the ability of exogenous erythropoietin to stimulate cognitive functions of animals after ischemia is actively explored. As cognitive functions are the ones mostly damaged by ischemic stroke, it has been suggested that the addition of erythropoietin receptor agonist will not only contribute to survival of the cells, but also improve these functions in animals who underwent ischemia. The aim of this work is to study the effect of erythropoietin receptor activation (EPOR) with Carbamylated darbepoetin (CdEPO) (PHARMAPARK LLC, Russia)

on behavior and memory of animals in normal condition and after ischemia. For this experiment, we used C57BL/6 mice. The studied mice were divided into two groups: with a single and triple entry interval (2 hours) by injection of CdEPO for investigation of the impact on the intact animals cognitive function. The animal behavior was tested on the first day after the injection. Preservation of basic activity and memory was studied with Open Field LE800S test, Shuttle Box LE916 (PanLab/Harvard Apparatus), "novel *object recognition*" *and* "puzzle box" test. CdEPO was injected after intravenously in 1, 3, 6, 12, 24, 48 and 6,12,24,48,72 hours after model of transient occlusion of the middle cerebral artery (tMCAO) (one-hour exposure) at 50 mkg/kg dose. Behavior was studied on the 4-th and 20-th postoperative day. The number of mice in the group was 10.

Single injection of CdEPO results in increasing of the freezing reaction duration. The duration of the grooming reaction increased regardless of the route of injection. Regardless of injection scheme, CdEPO positively affects the cognitive functions of animals: the time of study of a new object is increased. In the puzzle box mice were introduced into the brightly lit zone and should quickly move to the preferable dark zone through a tunnel. When the hole is closed with a bung, a single injection of CdEPO leads to decrease the action time. MCAO caused a disruption of the functional state of the animals. The injection of CdEPO in the first 6 hours after occlusion effectively restored the motor activity of mice and reduced the level of stress. The injection of CdEPO after "therapeutic window" had a less pronounced effect on the recovery of the brain functions on the 4-th day after surgery. Despite of injection scheme, the long-term memory in the "conditioned reflex of passive avoidance" test is restored for the group with active EPORs. At the same time, results of the "study of a new object" test demonstrated growth up of the associative learning. On the 20-th day, regardless of the injection scheme, mice motor activity restored, the stress level decreased and memory was preserved to intact values.

The ongoing research demonstrates the possibility of using the heterodimer EPORs activation as a promising strategy for the stimulation of memory and learning in post-ischemic period and in as well as for healthy organisms.

P II - 3-11

Peptide-agonist of protease-activated receptor 1 as modulator of photothrombosis-induced damage to mouse brain

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Activated protein C (APC), a serine protease of hemostasis, can be a potential component of post-stroke therapy, since it demonstrates cytoprotective and anti-inflammatory effects by cleavage of protease-activated receptor 1 (PAR1). Peptides-analogues of a tethered ligand of PAR1 released by APC, such as a new peptide AP9 can also have neuroprotective effects on brain cell cultures. For this reason, the study of the properties of AP9 in ischemia model *(in vivo)* is especially important.

Photothrombosis was carried out on male BALB/c mice. Injection of AP9 (i.v.) was performed once 10 min before the thrombosis in the doses of 0.2, 2 and 20 mg/kg, and twice – 10 min before and 1 h after (20 mg/kg). The volume of the lesion was assessed by magnetic resonance imaging (MRI), the blood-brain barrier permeability was evaluated by Evans blue extravasation. The number of GFAP+, Iba1+ cells and damaged neurons in the penumbra was calculated on histological sections by staining with specific antibodies, hematoxylin and eosin respectively. The level of motor deficiency was assessed in the tests "Cylinder" and "Gridwalk".

Using MRI, it was found that a single injection of AP9 significantly reduced the volume of lesion (69.9±8.2% relative to control) 24 h after thrombosis only at a dose of 20 mg/kg. At the same time, all used doses of AP9 did not alter either the percentage of damaged neurons or the neurological status of the animals.

Next, we evaluated the protective effect of double peptide administration at the most effective dose (20 mg/kg). It was shown that the repeated administration of AP9 led to a more pronounced reduction of the brain damage 24 h after ischemia (48.6±6.4%) compared to its single injection with further maintenance of the protective effect after 96 h (53.5±9.7%). Moreover, this peptide administration scheme provided a statistically significant improvement of the neurological status of the AP9-treated animals in the "Cylinder" test after 96 h (the relative contact time of the contralateral limb was 71.6±8.3%, control – 85.1±3%). In the "Gridwalk" test, AP9 decreased the number of motor errors more than 2 times compared to non-treated group 24 h after thrombosis. There was also a tendency to reduce the Evans blue leakage in the damaged hemisphere, but the peptide influence on the percentage of damaged neurons and the activation of astro-, microglia was not detected.

At the first time we demonstrated the protective properties of AP9 *in vivo* in ischemia model. The severity of lesion depends not only on the dose, but also on the multiplicity of peptide administration. The data of this study can serve as a basis for the formation of a new neuroprotective medicines.

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P II - 3-12

Post-stroke intranasal (+)-naloxone delivery reduces microglial activation and improves behavioral recovery from ischemic injury

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Questions: Inflammation has a major role in the pathophysiology of ischemic stroke. Microglia are the brain resident immune cells that are activated after ischemia but it is still unclear whether microglial activation is harmful or beneficial to the recovery. Both stereoisomers of naloxone, the (+) and (-) enantiomer, have been shown to decrease microglial activation. (-)-Naloxone is an opioid receptor antagonist while (+)-naloxone has only very low affinity for opioid receptors. Here we tested post-stroke delivery of naloxone for the ability to promote functional recovery after cortical stroke in rats. By using (+) enantiomer the possible side effects caused by opioid receptor antagonism can be avoided.

Methods: A unilateral cortical infarction was induced in adult male Sprague Dawley rats by transiently ligating the distal branch of the right middle cerebral artery with 10-0 suture and occluding both common carotid arteries for 60 minutes. At post-stroke day 1, the rats were balanced into groups based on neurological deficits and either (+)-naloxone (0.32 mg/kg; n=27), (-)-naloxone (0.32 mg/kg; n=7), vehicle (n=25) or no treatment (n=13) was given intranasally to the rats twice daily for the next 7 days. The behavioral recovery of the rats was followed for 2 weeks by body asymmetry test, Bederson's neurological score as well as horizontal and vertical activity. Microglial activation was quantified by immunohistochemical staining with anti-Iba1 antibody at post-stroke day 14. Infarct size and secondary neuronal loss in the thalamus were quantified using anti-NeuN staining at the same time-point. Data were analyzed with either one-way ANOVA followed by Bonferroni's post hoc test or Kruskal-Wallis nonparametric ANOVA followed by Mann-Whitney U test, or with Student's t-test or Mann-Whitney U test in the case of only two groups.

Results: On post-stroke days 10 and 14, (+)-naloxone as well as (-)-naloxone decreased body asymmetry ($p<0.001$ and $p=0.002$, respectively, for (+)-naloxone; $p=0.001$ and $p<0.001$, respectively, for (-)-naloxone) and Bederson's score ($p<0.001$ for (+)-naloxone; $p=0.002$ and $p=0.005$, respectively, for (-)-naloxone) compared to vehicle/no treatment. In addition, (+)-naloxone increased horizontal activity on day 14 post-stroke ($p=0.033$). (+)-Naloxone

decreased the infarct size by approximately 40% (p=0.019) and prevented secondary neuronal loss in the thalamus (p=0.003). (+)-Naloxone reduced the number of Iba1+ cells by 50% in the ipsilateral striatum (p=0.001) and thalamus (p<0.001) compared to vehicle/no treatment.

Conclusions: Post-stroke intranasal (+)-naloxone treatment decreased neuronal loss, reduced inflammation, and simultaneously promoted recovery from ischemic brain injury. Thus, repeated administration of naloxone during the phase of increasing microglial activation (i.e. days 1-7 post-stroke) could be a potential treatment for ischemic stroke.

P II - 3-14

The effects of R-phenibut in the experimental model of fluid percussion injury in mice

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Traumatic brain injury (TBI) is a leading cause of mortality and morbidity worldwide. There is an increased need for new treatment options for patients with TBI and associated symptoms. Our previous results have shown that in rats R-phenibut significantly improves histological outcome after transient middle cerebral artery occlusion. In R-phenibut treated animals a trend of recovery of tactile and proprioceptive stimulation was observed. After R-phenibut treatment at a dose of 50 mg/kg statistically significantly increased BDNF and VEGF gene expression in damaged brain hemisphere and these effects were thought to be related to the modulatory effects of the drug on the GABA-B receptor and α2-δ subunit of voltage-dependent calcium channel. The aim of the present study was to evaluate the effects of R-phenibut on neurobehavioral and histological outcomes following experimental traumatic brain injury.

Male *SW* mice were subjected to lateral fluid percussion (lFP) TBI. Two hours after the trauma, animals received an intraperitoneal injection of R-phenibut at doses of 10 and 50 mg/kg. After that R-phenibut was administered daily for an additional 7 days. The neurobehavioral status of mice was assessed on post-TBI days 1, 3 and 7 by the neurological severity score (NSS) testing. *Nissl* (cresyl violet) staining was used to assess the neuronal injury. *Nissl*-stained dark neurons (N-DNs) were investigated in the cerebral neocortex at the level of the cortical impact at day 7 after the lFP brain injury.

TBI induced significant functional deficits in TBI control mice as compared with sham-operated mice. The average NSS in TBI control group and sham-operated mice was 5.0 ±0.6 and 2.4±0.4, respectively. R-phenibut treatment at a dose of 50 mg/kg significantly ameliorated functional deficits and the average NSS in R-phenibut treated animals was 3.5 ±0.3 on the post-injury day 7. Histological analysis showed that R-phenibut treatment at a dose of 50 mg/kg significantly reduced the number of N-DNs in neocortex after TBI.

Our results provide evidence that R-phenibut reduces early neuronal injury, improves functional recovery and it might be used in clinical therapy in the acute phase after TBI.

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P II - 3-15

Does the death of brain pericytes contribute to no-reflow? A study on rat primary culture.

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Introduction: Recent findings suggest that rapid death and prolonged contraction (*rigor mortis*) of brain pericytes after ischemic stroke contributes to "no-reflow" following re-perfusion of the occluded artery, but this finding was later disputed. Since it is challenging to diff brain pericytes from vascular smooth muscle cells *in vivo*, we used primary culture of rat brain pericytes tp explore effects of oxygen glucose deprivation (OGD) on cell viability and on parameters that indicate contractility of the cells.

Methods: Brain pericytes from Sprague-Dawley rats were cultured, purified and then exposed either to OGD or to control conditions for 2-24h. Cell viability and apoptosis were estimated by flow cytometry. In some cases, cells were imaged in a cell observer (5 min intervals over 15h) before and after 20min, 1h or 3h OGD protocols. Stacks of images of randomly selected sets of 11-22 cells from different flasks for each experiment were analyzed using a self-written software (available at http://www.synosoft.de) to assess differences in the single cell membrane mobility (SCMM) and in the two parameters that served to assess cell processes" length: the area/perimeter ratio (APR) and fractal dimension (FD) for the same cells before and after OGD protocols. A two-way ANOVA was conducted to determine the effects of OGD in the presence or absence of cytokines presence and of duration of incubation on viability of the cells. The paired and unpaired t-tests were used to analyze significance of differences in SCMM and parameters of contractility, respectively.

Results: There was no significant reduction in cell viability or increase in apoptosis in cultures that were exposed to 2h and 6h OGD protocols. Major and significant ($p<0.001$) reduction in cell viability and increase in apoptosis occurred after the 24h OGD protocol; these detrimental effects were largely removed when erythropoietin, vascular endothelial growth factor and angiopoietin 1 were added together to the cell culture medium, but not when either of these cytokines was added separately. Twenty minutes and 1h OGD protocols caused a significant reduction in SCMM ($p<0.05$ and $p<0.01$, respectively). There was no significant difference in SCMM values before and after the 3h OGD protocol ($p>0.05$). There was a significant increase in APR after 20min of the OGD protocol ($p<0.01$), while a significantly decreased APR ($p<0.05$) and a significantly increased FD ($p<0.01$) were seen after 3h of the OGD protocol.

Conclusions: Significant cell death occurred only after 24h OGD protocol; this was largely prevented when cytokines that are released by the neurovascular unit during hypoxia were added to the medium. Pericytes contracted after 20min and 1h of the OGD protocol, while after 3h of the OGD protocol they were not contracted more than before the OGD protocol. Thus, we found no evidences that brain pericytes died rapidly or remained contracted during exposure to the OGD protocols.

P II - 3-16

Identification of novel neuroprotective drug combinations for the treatment of ischemic stroke through a systems biology-based drug repositioning approach

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Introduction: Ischemic stroke is a leading cause of morbidity and mortality worldwide. Beyond the standard thrombolytic therapies, there is still no effective treatment to mitigate or even reverse the progression of stroke disease. Since potential neuroprotective treatments from pre-clinical studies have failed to translate into clinical success, effective neuroprotective drug discovery is still urgently needed. Combinational treatment approaches are emerging as powerful strategies to synergistically and simultaneously target more than one disease-response mechanism underlying complex pathologies such as stroke. In this line, we hypothesize that the individual

modulation of a single pathological mechanism might not be sufficient to attenuate the progression of stroke, so we aimed to identify combinations of FDA-approved drugs with potential neuroprotective effect for stroke treatment.

Methods: We used a systems biology-based technique relying on artificial intelligence and pattern recognition models to integrate available biological, pharmacological and medical knowledge into mathematical models that simulate *in silico*the complex behavior of stroke disease. Novel brain proteomics and transcriptomics data obtained from deceased ischemic stroke patients were used to enrich and curate the emergent mathematical models. Drug repositioning solutions were acquired by perturbing the stroke-mimicking model with multiple sets of stimuli, which corresponded to two-by-two combinations of drugs from the DrugBank Database. Finally, we experimentally tested the neuroprotective effect of two drug combinations in a mouse model of transient cerebral ischemia.

Results: More than 5 million drug combinations were evaluated for their neuroprotective effect in our mathematical models. Approximations to the best treatment solution for ischemic stroke were obtained establishing a threshold of minimum predicted probability to exert neuroprotection of the ischemic brain of 80%. This cut-off point corresponded to the maximum predictive capacity obtained from screening in our mathematical models a set of previous neuroprotective treatments tested in clinical trials for ischemic stroke without success. Finally, the therapeutic effects of two promising drug combinations were experimentally validated in a mouse model of transient cerebral ischemia, while individual drug administration showed no effect.

Conclusions: We have identified potential neuroprotective combinations of FDA-approved drugs by using a systems biology-based simulation of the complex behavior of stroke disease. The simultaneous modulation of different motives and pathways altered after ischemic stroke seems a good strategy to mitigate the progression of the disease.