Fifteenth International Symposium

on

"Signal transduction in the blood-brain barriers"

Programme and Abstracts

Botanical Garden, Park Sanssouci

Potsdam/Germany, September 13-16, 2012

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Programme

Thursday, September 13, 2012

09.30 - 11:30	*Registration (Steigenberger Hotel, Allee nach Sanssouci 1, 14471 Potsdam)*
12:15 - 12:45	*Registration (Lecture Hall, Botanical Garden, Maulbeerallee 2a, 14469 Potsdam)*
12.30	*Attachment of posters (poster rooms)*
13.00	Introductory remarks (Lecture Hall)
13.05	Welcome by Local Representatives
13:10	Introductory Lecture: E. Dejana, Milan/Italy: Physiological and pathological determinants of blood-brain barrier properties

Session I: Drug delivery to the brain

Chairs: B. Engelhardt, W. Banks

13:45	H.-J. Galla, Münster/Germany: Nanoparticles: A vehicle for transport across the blood-brain barrier
14:05	J. Nicolazzo, Parkville/Australia: Altered blood-brain barrier transport of therapeutic agents in the 3xTg mouse model of Alzheimer's disease
14:25	R. Gabathuler, Vancouver/Canada: Incorporation of Transcend (melanotransferrin, MTf or p97) in biologics allows their transport across the BBB for the treatment of brain disorders
14:45	D. Begley, London/UK: Transcytosis at the BBB: A re-evaluation
15:05	NN, Short communication selected from poster contributions
15:15	*Coffee Break*

Session II: The BBB during development

Chairs: J. Abbott, D. Begley

15:45	C. Förster, Würzburg/Germany: Glucocorticoids and perinatal brain damage
16:05	H. Stolp, Oxford/UK: Vascular effects of developmental inflammation
16:25	P. Johansson, Munich/Germany: The transcription factor Otx2 regulates choroid plexus development and function affecting neural stem cells at distant sites via changes in CSF signalling
16:45	D. Virgintino, Bari/Italy: Differential expression patterns of chemokine CXCL12 and receptors CXCR4/CXCR7 are implicated in human brain development and vascularization
17:05	H.-C. Bauer, Salzburg/Austria: Transport in the choroid plexus in embryonic and adult mice
17:45	*Guided Tour (Orangerie Castle) -optional-*
20:00	*Informal Welcome Reception (Steigenberger Hotel)*

Friday, September 14, 2012

08:40 *Registration / Attachment of posters*

Session III: Regulation of the BBB
Chairs: E. de Vries, G. del Zoppo

09:00 A. Zochowska, Ann Arbor, MI/USA: ZO-1-VASP interaction controls tight junctional complex stability and function

09:20 S. Buch, Omaha, NE/USA: Role of Notch-1 signaling in cocaine-mediated PDGF-BB expression: Implication for disruption of brain endothelial cells

09:40 A. Nuttall, Portland, OR/USA: Role of STAT3 in the cochlear vascular barrier

10:00 M. Lopes Pinheiro, Amsterdam/NL: Sphingosine 1-phosphate receptor 5 mediates the immune quiescence of the human brain endothelial barrier

10:15 NN, Short communication selected from poster contributions

10:25 *Coffee break*

Session IV: BBB alterations during ischemia and oxidative stress
Chairs: M. Deli, R. Cecchelli

11:00 G. Bix, Lexington, KY/USA: The matrix reloaded: Perlecan domain V and the blood-brain barrier in stroke and Alzheimer's disease

11:20 G.J. del Zoppo, Seattle, WA/USA: The roles of β-1 integrins in endothelial tight junction and microvessel permeability barrier integrity during focal cerebral ischemia

11:40 P. Fraser, London/UK: Nrf2 quantified in endothelium of core and peri-infarct regions of rat brain following ischaemia-reperfusion

12:00 G.N. Shah, St. Louis, MO/USA: Attenuation of high glucose-induced oxidative stress and apoptosis in brain pericytes by a mitochondrial carbonic anhydrase inhibition

12:20 C. Bellmann, Berlin/Germany: The role of conserved cysteines in the cis- and trans- homooligomerization of occludin under oxidative stress

12:35 NN, Short communication selected from poster contributions

12:45 *Lunch break*

Session V: BBB alterations in brain diseases
Chairs: C. Förster, I. Krizbai

14:00 A. Friedman, Beer-Sheva/Israel: What does the blood-brain barrier protect the brain from?

14:20 K.-H. Plate, Frankfurt/M./Germany: Vascular homeostasis in malignant brain tumors

14:40 S. Niclou, Luxembourg/Luxembourg: The Side population phenotype in human glioblastoma is exclusively stroma-derived and its efflux properties are unaffected by anti-VEGF treatment

15:00 S. Fischer, Giessen/Germany: Role of extracellular RNA in regulating adhesion and transmigration of tumor cells at the blood-brain barrier

15:20 NN, Short communication selected from poster contributions

15:30 **Poster Session I**, *Coffee Break*,
Chairs: S. Fischer, A. Zochowska, P. Fraser, S. Liebner

Session VIa: BBB alterations in neurodegenerative diseases
Chairs: A. Friedman, J.-M. Heard

17:00 W. Banks, Seattle, WA/USA: Neuroinflammation inhibits abeta efflux: Implications for Alzheimer's disease

17:20 M. Toborek, Miami, FL/USA: Interactions of HIV-1 with amyloid beta at the blood-brain barrier level

17:40 C. Pietrzik, Mainz/Germany: Genetic evidence for LRP1 and PrPc mediated amyloid-β transcytosis across the blood-brain barrier

18:00 E. Stopa, Providence, RI/USA: ApoE, agrin, microvascular injury and blood-brain barrier compromise in sporadic (late onset) Alzheimer's disease

18:20 NN, Short communications selected from poster contributions

18:40 *Business meeting (information on symposium organization 2013)*

19.00 *Barbeque*

Saturday, September 15, 2012

8.40 *Registration / Attachment of posters*

Session VIb: BBB alterations in neurodegenerative diseases
Chairs: C. Pietrzik, G. Bix

09.00 M. Scarpa, Padova/Italy: Pathophysiology of lysosomal storage diseases as model for neurodegenerative diseases

09:20 J.-M. Heard, Paris/France: Biological markers of severity in lysosomal storage disease

09:40 E. de Vries, Amsterdam/NL: microRNAs control brain endothelial cell barrier function and immune quiescence, implications for MS

10:00 A. Armulik, Zurich/Switzerland: Pericyte-regulated functions of the BBB

10:20 NN, Short Communication selected from poster contributions

10:30 *Coffee break*

Session VIIa: Inflammation at the BBB
Chairs: M. Scarpa, Y. Persidsky

11:00 B. Engelhardt, Bern/Switzerland, The blood-brain barrier: Checkpoint Charlie for immune cell entry into the CNS

11:20 E. Solito, London/UK: Leukocyte-endothelial cell cross talk at the blood-brain barrier: The good and bad side of a two way system

11:40 R. Lyck, Bern/Switzerland: T cell extravasation across the blood-brain barrier endothelium: Differential contribution of ICAM-1, ICAM-2, VCAM-1 and ALCAM to T cell adhesion, crawling and diapedesis

12:00 D. Cribbs, Irvine, CA/USA: Mixed cerebrovascular disease: Role for hypertension in cerebral amyloid angiopathy, microhemorrhages and neuroinflammation

12:20 NN, Short communication selected from poster contributions

12.30 *Lunch break*

Session VIIb: Inflammation at the BBB

Chairs: D. Virgintino, T. Tenenbaum

13:45 Y. Persidsky, Philadelphia, PA/USA: Novel approaches for blood-brain protection in neuroinflammation

14:05 H. Wolburg, Tübingen/Germany: The involvement of the blood-CSF barrier in the african trypanosomiasis

14:25 P.-O. Couraud, Paris/France: CD147 is the receptor for pilus-mediated adhesion of meningococci to brain vascular endothelium

14:45 G. Kunis, Rehovot/Israel:A two-signal model for the activation of the blood-CSF barrier for immune cells trafficking

15:05 NN, Short Communications selected from poster contributions

15:15 **Poster Session II** */ Coffee Break*

Chairs: A. Keller, R. Lyck, H.-C. Bauer, M.Toborek

Session VIII: Transport processes at the BBB

Chairs: M. Hammarlund-Udenaes, H.-J. Galla

17:00 G. Fricker, Heidelberg/Germany: Regulation of transporter proteins of the blood-brain barrier by nuclear receptors

17:20 M. Tachikawa, Sendai/Japan: Pathophysiological impact of hemichannels on the blood-brain barrier transport

17:40 V. Makrides, Zurich, Switzerland: Blood-brain barrier endothelial amino acid transporters in the control of brain interstitial amino acid homeostasis

18:00 M. Jablonski, Philadelphia, MA/USA: Astrocytes dysregulate BBB integrity and ABC transporter properties in ALS, a neurodegenerative disease of the motor system

18:20 J. Pahnke, Magdeburg/Germany: A new pathogenic pathway and causative treatment option for Alzheimer's disease – from aging to mitochondrial and ABC transporter dysfunction

20.00 *Symposium Dinner (Steigenberger Hotel)*

Sunday, September 16, 2012

Session IX: Novel techniques and models to assess BBB functions
Chairs: G. Fricker, T. Kleine

09.00	J. Abbott, London/UK: Optimisation and functional characterisation of a new porcine brain endothelial cell model of the BBB
09:20	R. Cecchelli, Lens/France: Assessing the free brain/free plasma ratio in vitro in early drug discovery
09:40	M. Deli, Szeged/Hungary: Comparison of epithelial cell line-based surrogate and brain endothelial cell-based blood-brain barrier models for drug screening
10:00	C. Schwerk, Mannheim/Germany: Cellular response to *Neisseria* meningitidis in a Human Model of the blood-cerebrospinal fluid barrier
10:15	T. Tenenbaum, Mannheim/Germany: Infection with echovirus 30 - effects on leukocyte migration across the blood-cerebrospinal-fluid barrier in a human in vitro model
10:30	*Coffee break*

Session X: Structure, function and regulation of tight junction proteins
Chairs: P.-O. Couraud, H. Wolburg

11:00	S. Liebner, Frankfurt/M./Germany: Molecular regulation of endothelial blood-brain barrier function in health and disease
11:20	J. Cording, Berlin/Germany: Detection of tight junction strand morphologies of claudins and tight junction associated marvel-proteins
11:40	K. Beyenbach, Ithaka, NY/USA: The structure and function of septate junctions
12:00	A. Szczepkowska, Poznan/Poland: PCB153 affects tight junction proteins in ovine choroid plexus
12:20	J. Rossa, Berlin/Germany: Elucidating the molecular organization of tight junctions strands
12:40	Concluding remarks
13.00	*End of symposium*

Poster Presentations

Group I (presentations displayed on September 13-14, 2012)

I-1 Baruch, K. (Rehovot, Israel): Functional aging of the brain reflects epithelium-T cell crosstalk at the blood-CSF barrier

I-2 Bénardais, K. (Hannover, Germany): In vivo and in vitro effects of Nrf-2 inducing substances on blood-brain barrier tight junction proteins

I-3 Benson, K. (Münster, Germany): The impact of extracellular matrices on the barrier function of cerebral endothelial cells

I-4 Blecharz, K. (Berlin, Germany): The properties of brain endothelial cells in an in vitro model of Moyamoya disease

I-5 Blomqvist, A. (Linköping, Sweden): Lipopolysaccharide-induced fever depends on prostaglandin E2 production specifically in brain endothelial cells

I-6 Breitkreuz-Korff, O. (Berlin, Germany): Alternative approach for treatment of metachromatic leukodystrophy disease with enzyme replacement therapy by crossing the blood-brain barrier

I-7 Dabrowski, S. (Berlin, Germany): Modulation of paracellular barrier properties using claudin-mimetic proteins and peptides

I-8 Ercal, N. (Rolla MO, USA): Oxidative stress in the blood-brain barrier induced by numerous toxins and its consequences

I-9 Evans, M. (Oxford, UK): CNS targeted anti-inflammatory agent reduces pathology in mouse model of ALS

I-10 Fallier-Becker, P. (Tübingen, Germany): An allograft glioma model reveals the dependence of aquaporin-4 expression on the brain microenvironment

I-11 Han, H. (Daegu, Korea): Oleic acid increases permeability of blood-brain barrier in the rat brain

I-12 Harrer, A. (Salzburg, Austria): Natalizumab effects behind CNS barriers: Reduced intrathecal IgG synthesis in the cerebrospinal fluid from MS patients on Natalizumab therapy

I-13 Janson, B. (Ludwigshafen, Germany): The aged rat – a model for Alzheimer's disease?

I-14 Kaya, M. (Istanbul, Turkey): Repetitive hyperthermia-induced seizures in early life alter blood-brain barrier integrity and seizure thresholds in rats with cortical dysplasia

I-15 Michalec, K. (Warsaw, Poland): Regulation of L-carnitine transport through the blood-brain barrier by protein kinase C activation

I-16 Mirshafiey, A. (Tehran, Iran): The molecular mechanism of blood-brain barrier alterations in neurodegenerative diseases

I-17 Neuhaus, W. (Würzburg, Germany): Inhibition of OGD-induced and astrocyte enhanced blood-brain barrier breakdown by specific receptor modulators

I-18 Nußhold, C. (Graz, Austria): MPO-derived 2-chlorohexadecanal as effector of BBB function in vitro and in vivo

I-19 Skipor, J. (Olsztyn, Poland): Analysis of quercetin derivatives in the cerebrospinal fluid of adult ewes

I-20 Sparaneo, A. (Bari, Italy): Aquaporin-4 ablation impairs blood retinal barrier permeability

I-21 Töpfer, S. (Salzburg, Austria): Plasticity and differentiation potential of porcine cerebral capillary endothelial cells in vitro

I-22 Tóth, A. (Szeged, Hungary): Amyloid-beta 1-42 petide-induced toxicity in the cells of the neurovascular unit: Protection by docosahexaenoic acid
I-23 Touré Ndouo, F. (Franceville, Gabon): Pathogenicity of *Plasmodium falciparum* field isolates: identification of the new immunological and therapeutic targets for severe malaria
I-24 Üllen, A. (Graz, Austria): Phloretin ameliorates MPO-mediated barrier dysfunction in brain microvascular endothelial cells
I-25 Zhou, Q. (Chongqing, China): Affect of simulated high altitude environment exposed to the BBB permeability and sodium aescinate to protective role of BBB and anti-leakage mechanism under hypoxia

Group II (presentations displayed on September 15-16, 2012)

II-1 Abadier, M. (Bern, Switzerland): T cell diapedesis across the blood-brain barrier endothelium: The inflammatory stimulus regulates the trans- versus the paracellular pathway
II-2 Bayat, Z. (Quchan, Iran): Computational approach to the prediction of blood-brain barrier permeability using density functional theory
II-3 Blasig, R. (Berlin, Germany): Effect of hypoxic conditions and presence of caprate on claudins in isolated murine brain capillaries
II-4 Bromander, S. (Gothenburg, Sweden): Central inflammatory markers in response to surgical stress and in relationship to personality
II-5 Coisne, C. (Bern, Switzerland): Investigating claudin-3 and claudin-5 functions at the blood-brain barrier
II-6 Garcia Polite, F. (Barcelona, Spain): Software to generate cad of brain capillary network for FEM simulation
II-7 Goñi de Cerio, F. (Vizcaya, Spain): Up regulation of different blood-brain barrier in vitro models by astroglia
II-8 Helms, H. (Copenhagen, Denmark): In vitro evidence for the brain glutamate efflux hypothesis; brain endothelial cells co-cultured with astrocytes display a polarized brain-to-blood transport of glutamate
II-9 Kleine, T. (Marburg, Germany): Signal transduction to human central nervous system (CNS) becomes modulated with blood-brain barriers, uncovered with the Marburg cerebrospinal fluid (CSF) model
II-10 Krizbai, I. (Szeged, Hungary): Differential response to stress induced analgesia in two mouse strains: a role for the BBB?
II-11 Labus, J. (Berlin, Germany): The role of beta 1 integrins in a novel in vitro blood-brain barrier model
II-12 Mack, A. (Tübingen, Germany): Tight junctions form barriers in the retinal nerve fiber layer of teleost fish
II-13 Michalak, S. (Poznan, Poland): Circulating tight-junction proteins as predictors of clinically evident hemorrhagic transformation in ischemic stroke patients
II-14 Protze, J. (Berlin, Germany): Determinants contributing to claudin barrier and ion channel formation
II-15 Ruszkowski, P. (Poznan, Poland): Influence of valproic acid on Temozolomide transport through blood-brain barrier in vitro model
II-16 Schoknecht, K. (Berlin, Germany): Quantitative assessment of blood-brain barrier dysfunction and cell damage *in vivo* after cortical photothrombosis
II-17 Stalmans, S. (Ghent, Belgium): Exploring the Brainpeps database

II-18 Steinmann, U. (Mannheim, Germany): Transmigration of polymorphonuclear neutrophils and monocytes through the human blood-cerebrospinal fluid barrier after bacterial infection in vitro

II-19 Tscheik, C. (Berlin, Germany): Sodium caprate transiently opens claudin-5-containing barriers at tight junctions of epithelial and endothelial cells

II-20 Veshnyakova, A. (Berlin, Germany): cCPE as a potential tool to affect claudins, present in the BBB

II-21 Veszelka, S. (Szeged, Hungary): Mono-, double and triple co-culture models of the blood-brain barrier: A gene-array study

II-22 Walter, F. (Szeged, Hungary): Blood-brain barrier changes in ornithine-induced acute pancreatitis model

II-23 Willis, C. (Biddeford ME, USA): Adherens junctions and extracellular matrix remodeling form a size selective barrier following focal astrocyte loss and post-translational occludin modification

II-24 Yusof, S. (Penang, Malaysia): Applications of an improved porcine brain endothelial cell (PBEC) model of the BBB

Nanoparticles: vehicles to facilitate transport across the blood-brain barrier

Hans Joachim Galla; Institute for Biochemistry, University of Münster, Wilhelm Klemm Str 2, D-48149 Münster, Germany

Nanoparticles have been widely used as carriers to transfer drugs across the blood brain barrier. Following this approach we used polysorbate 80(PS80)-coated poly(n-butylcyano-acrylate) nanoparticles (PBCA-NP) To allow such an application it is important to ascertain their effect on the BBB integrity. This has been investigated by monitoring the development of the transendothelial electrical resistance (TEER) after the addition of PBCA-NP employing impedance spectroscopy porcine in vitro model. Additionally, the integrity of the BBB in vitro was verified by measuring the passage of the reference substances 14C-sucrose and FITC-BSA after addition of PBCA-NP. We showed that the application of PS80-coated PBCA-NP leads to a reversible disruption of the barrier within 4 hs in the TEER experiment confirmed by 14C-sucrose and FITC-BSA permeability studies. The barrier disruption recovered completely within the next 10-15 hours. These results indicate that PS80-coated PBCA-NP might be suitable for the use as drug carriers. The reversible disruption also offers the possibility to use these particles as specific opener of the BBB. Instead of incorporating the therapeutic agents into the NP, the drugs may cross the BBB after being applied simultaneously with the PBCA-NP.

In a second approach I will report the use chemically modified iron oxide nanoparticles to cross the BBB. Fe_3O_4 is used as contrast agents in MRI diagnosis. It will be shown that nanoparticles functionalized by lactoferrin are transferred to the brain by receptor-mediated transcytosis. An excellent in vivo/in vitro correlation was found in comparison to MRI performed on rats. This clearly demonstrates the useful application of reliable in vitro BBB models.

Rempe R, Cramer S, Hüwel S and Galla HJ (2011) Transport of Poly(n-butylcyano-acrylate) nanoparticles across the blood–brain barrier in vitro and their influence on barrier integrity; Biochim.Biophys.Res.Commun.406,64-69

Cramer S, Rempe R and Galla HJ (2012) Exploiting the properties of biomolecules for brain targeting of nanoparticulate systems , Cur Med Chem 19, 3163-3187

Qiao R, Jia Q, Hüwel S, Xia R, Liu T, Gao F, Galla HJ and Gao M; (2012); Receptor-mediated delivery of magnetic nanoparticles across the blood-brain barrier; ACS Nano 4,3304-3310

Altered blood-brain barrier transport of therapeutic agents in the 3xTg mouse model of Alzheimer's disease

Joseph Nicolazzo; Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia

The blood-brain barrier (BBB) has been suggested to be altered in Alzheimer's disease (AD) with reports of inter-endothelial cell tight junction dysfunction, reduced expression of the efflux transporter P-glycoprotein (P-gp) and cerebrovascular basement membrane thickening. It remains unknown whether such changes impact on the brain entry of drugs, potentially placing AD patients at increased risk of drug-induced neurotoxicity. Therefore, the aim of this study was to evaluate the impact of AD on the BBB transport of drugs with different mechanisms of transport. Radiolabelled marker compounds were transcardially perfused (0.5 µCi/mL at 2 mL/min for 4 min) in 18 month male wild-type (WT) and 3xTg AD mice. The marker compounds were ^{14}C-sucrose (paracellular marker), ^{3}H-diazepam and ^{3}H-propranolol (passive transcellular markers) and ^{3}H-digoxin, ^{3}H-loperamide and ^{3}H-verapamil (P-gp substrates). Following perfusion, the cortex and hippocampus were dissected, analysed for radioactivity and cortex-to-perfusate (C:P) and hippocampus-to-perfusate (H:P) ratios determined. The C:P ratio of ^{14}C-sucrose was 0.021 ± 0.003 (mean ± SD) in WT mice and 0.026 ± 0.005 in 3xTg mice, and similarly, the H:P ratios of ^{14}C-sucrose were 0.019 ± 0.004 and 0.023 ± 0.004 in WT and 3xTg mice, respectively. The BBB transport of P-gp substrates was not significantly affected in AD mice, whereas the BBB transport of passive transcellular markers was significantly ($p<0.05$) reduced in these mice (C:P and H:P ratios in 3xTg AD mice being 2.2-2.3 fold lower for ^{3}H-diazepam and 1.9-2.7 fold lower for ^{3}H-propranolol). These studies demonstrate that the brain exposure of drugs can be significantly affected during AD, depending on their mechanism of BBB transport. Passive transcellular diffusion of drugs across the BBB is decreased in AD, likely due to cerebrovascular membrane thickening, and this appears to be counteracted for P-gp substrates, likely as a result of the reduced BBB expression of P-gp in AD.

Incorporation of Transcend (melanotransferrin,MTf or p97) in biologics allows their transport across the BBB for the treatment of brain disorders

Reinhard Gabathuler[1], Timothy Z. Vitalis[1], Umar Iqbal[3], Maria Moreno[3], Wilfred A.Jefferies[2]; [1]biOasis Technologies Inc., Vancouver, BC; [2]University of British Columbia, Vancouver, BC, [3]NRC-Institute of Biol. Sci., Ottawa, ON, CANADA

The blood-brain barrier (BBB) is formed by brain capillary endothelial cells characterized by tight junctions between cells and a high expression of efflux pumps only allowing brain access to nutrients necessary for cell survival and function. These properties of the BBB result in the incapacity of small and large therapeutic compounds to reach the brain in therapeutic concentrations. Research has been necessary for the development of new peptide and protein vectors able to cross the BBB and able to deliver therapeutic agents in therapeutic concentrations to the brain.

Transcend (Melanotransferrin-MTf, p97) has been developed by biOasis Technologies Inc. as a vector for receptor mediated drug delivery into the brain involving a receptor of the family of LDL receptor related protein (LRP). Using MTf-rhodamine, we have shown by fluorescence microscopy that MTf are rapidly transported in the brain parenchyma, colocalize with markers of neurons and astrocytes and endocytosed in endosomes and lysosomes and Transcend has been shown to transport a small anti-cancer agent doxorubicin across the BBB to brain tumors in therapeutic concentration.

In a proof of concept study we demonstrate that antibodies labelled with rhodamine or other fluorescent dyes can be transported in the brain parenchyma after incorporation of Transcend. Using marker proteins labelled with fluorescent dyes and binding to lectins or to CD31 localized specifically on brain capillary endothelial cells separation of the proteins localized in the brain parenchyma and capillaries can be done. By quantitative confocal fluorescence microscopy we determined that 10 to 15 times more antibodies were delivered in the brain parenchyma when conjugated to Transcend. Data on two antibodies conjugated to Transcend will be presented an antibody recognizing Her-2 and an antibody recognizing beta-amyloid peptides.

These studies demonstrate that Transcend can be used as a vector for the transport of biologics such as antibodies across the BBB and capable of shuttling therapeutic levels of a variety of compounds from small anti-cancer agent to larger biologics such as antibodies across the BBB for the treatment of neurological disorders.

ApoE and Apo A-1 target albumin nanoparticles to brain neurones *in vivo*.

David J. Begley, Institute of Pharmaceutical Science, Kings College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK.

Human serum albumin nanoparticles, covalently bound, via a PEG spacer, to either apolipoprotein E or apolipoprotein A-1 as targetors, were injected intravenously into either SV129 mice or Wistar rats, under general anaesthesia. The animals were sacrificed after 15 or 30 minutes and the brains perfusion fixed and examined by electron microscopy. Only nanoparticles targeted with the apolipoproteins were detected in brain endothelial cells, transcytosed and subsequently appeared in the cytoplasm of neurones. No uptake into the brain was detected with nanoparticles only bearing the PEG spacer [1, 2]. Tight junction integrity, as determined with ionic lanthanum, was maintained during the experiments. Within the brain, nanoparticles could be observed in all brain regions examined, both in the cytoplasm of endothelial cells and brain cells including neurones. The only particles convincingly within brain extracellular space were seen in the extracellular matrix surrounding the capillaries. The pathway of transfer of the particles to the neurones is still unclear as the particle diameter 200-250nm is too large for them to move freely in the brain extracellular space which has an estimated width of 38-64nm [3].

[1] Zensi A., Begley D., Pontikis C., Legros C., Mihoreanu L., Wagner S., Büchel C., von Briesen H., Kreuter J. (2009) Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones. J Contr Rel. **137**: 78-86. (2009)

[2] Zensi A., Begley D., Pontikis C., Legros C., Mihoreanu L., Büchel C., Kreuter J. Human serum albumin nanoparticles modified with apolipoprotien A-1 cross the blood-brain barrier and enter the rodent brain. J Drug Target **18**: 842-848. (2010)

[3] Thorne RG. and Nicolson C. In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. Proceedings of the National Academy of Sciences USA. **103**: 5567-5572. (2006)

Effects of synthetic glucocorticoids on fetal and newborn BBB maturation: implications in adverse health consequences

Winfried Neuhaus and Carola Förster; Abteilung Experimentelle Anästhesiologie, Klinik und Poliklinik für Anästhesiologie, Universitätsklinikum Würzburg

Corticosteroids are among the most powerful drugs used in the perinatal and neonatal period, the merits and risks of antenatal as well als postnatal steroid administration are however controversial. While a single course of antenatal corticosteroids in women at risk of premature labour has been shown to effectively reduce respiratory distress syndrome, intraventricular haemorrhage and neonatal mortality as well as neurodevelopmental disorders like cerebral palsy, the practice of multiple courses of corticosteroids has been associated with neurological impairment and reduction in birth weight, lung weight and growth. Moreover, the postnatal systemic administration of corticosteroids have been related to several short-term side-effects, an increased risk of neurodevelopmmental disability, and particularly cerebral palsy in survivors besides showing benefits in reducing chronic lung disease. In our study, the long-term neurodevelopmental outcome of both multiple courses of antenatal corticosteroids, as well as postnatal corticosteroid therapy was related to the development of the glucocorticoid-exposed non-adult blood-brain barrier (BBB). A special emphasis was put on structural changes in BBB cell biology and transport protein expression patterns.

Vascular effects of developmental inflammation

Helen Stolp[1], Matt Evans[2], Zoltan Molnar[1], Daniel Anthony[3]; [1]Department of Physiology, Anatomy and Genetics; [2]Nuffield Department of Clinical Neurosciences; [3]Department of Pharmacology, University of Oxford, UK

Inflammation has been implicated in the pathogenesis of a number of neurological disorders. This includes neurodevelopmental disorders, where long-term behavioural changes are observed following early life inflammatory insult. It is currently unclear how systemic inflammation reaches the developing brain; however, changes in blood-brain barrier permeability have been implicated. This work aims to determine the signalling of the blood vasculature and choroid plexus following inflammation and to correlate changes in vascular function with barrier permeability. Central inflammation was produced in C57B/6 mice using an intrastriatal injection of rIL-1beta (1ng) at postnatal day (P) 7, 14 and 21. Barrier permeability was determined by immunohistochemistry for endogenous plasma proteins within the brain parenchyma. The presence of white blood cells in the brain was also determined using immunohistochemistry. These measures were correlated with markers of barrier function (claudin-5, occludin) and vascular immune activation (VCAM-1, ICAM-1, TLR4, IL-1beta, TNF-alpha, IL-6) using both immunohistochemistry and qRT-PCR. Brain injury associated with vascular changes will be assessed using activate caspase-3 and markers of gliosis (GFAP and Iba1 immunohistochemistry). CNS inflammation caused an age-dependent breakdown in blood-brain barrier permeability and a substantial neutrophil recruitment to the affected brain areas at P14. At P21 substantial up-regulation of vascular signalling pathways were observed, including an up-regulation of ICAM-1, but this was not associated with a substantial change in blood-brain barrier permeability. This study has demonstrated an age-specific response of the brain vasculature to central inflammatory insult, which is controlled by a complex signalling mechanism.

The transcription factor Otx2 regulates choroid plexus development and function affecting neural stem cells at distant sites via changes in CSF signalling

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Despite the key role of the choroid plexus in secretion and composition of cerebrospinal fluid (CSF) still little is known about its specification and role during development. Here we demonstrate a key role for the transcription factor Otx2 in development of all four choroid plexuses. Deletion of Otx2 by the Otx2CreERT2 driver-line at E9 resulted in lack of all choroid plexuses, whereas deletion by the Gdf7-Cre driver-line affected predominately the hindbrain choroid plexus. Further analysis of the latter embryos revealed altered expression of Wnt-signaling components in the hindbrain choroid plexus and alterations in CSF composition. This, surprisingly, lead to a region-specific effect on the proliferation of the stem cells in the distant cerebral cortex caused by alterations in Wnt-signaling. Taken together, our results reveal a key regulator of choroid plexus development and thereby unravel the role of choroid plexus in long-distance signaling via the CSF.

Differential expression patterns of chemokine CXCL12 and receptors CXCR4/CXCR7 are implicated in human brain development and vascularization

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During brain development, vascularization of the cerebral cortex occurs according to sprouting mechanisms that start at the perineuronal plexus, encompass the forming cortex, and proceed in subcortical layers with the formation of new capillary loops. Growing vascular sprouts and newly formed microvessels show a blood-brain barrier (BBB) profile, revealed by the endothelial expression of tight junction proteins and metabolic and efflux transporters. During vascular sprouting and BBB differentiation, endothelial cells (ECs), pericytes, radial glial cells (RGCs) and astrocytes co-participate in the process and, through the expression of several regulating factors, accomplish multiple tasks in the vessel growth and BBB differentiation program. Evidence of a role of chemokines in vascular growth during normal development and in tumors has been reported. Our work is focused on the chemokine CXCL12, also known as stromal-derived factor-1 (SDF-1) and on its receptors CXCR4 and CXCR7, in view of their roles in cell migration and differentiation, particularly in CNS development and vascularization. The cell expression and localization of CXCL12 and its receptors were revealed by immunofluorescence confocal microscopy on human developing brain at midgestation. At this time CXCL12 identifies RGC subtypes and cerebral cortex radial astrocytes, appears asymmetrically distributed in perivascular astrocytes of subcortical layers, and marks ECs of angiogenically activated, growing microvessels, while CXCR4 and CXCR7 are expressed in migrating neuroblasts and in ECs and pericytes of vascular sprouts. Secreted CXCL12 has been demonstrated in the extracellular space in monomeric or dimeric forms that differentially activate CXCR4 and CXCR7 pathways [1]; CXCR7 regulates CXCR4 signalling and also controls ligand availability [2]. According to these data, the present results support the idea that the CXCL12/CXCR4/CXCR7 axis, which appears directly involved in brain vascularization, may be implicated in regulating both microvessel growth and BBB differentiation through differential expression patterns and different ligand effects on cell functions.

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Protein transport from blood to CSF across the choroid plexus of embryonic and adult mouse brain

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Introduction: The understanding of brain development is to a great part depending on determining to what extent barrier mechanisms are junctional and whether there are barrier mechanisms that are specific to the developing brain. There is now evidence that fundamental barrier properties are present very early in development. It has been shown earlier that numerous blood–brain barrier-.related genes are expressed in the developing brain, and recently we have described the transcriptosome of embryonic and adult mouse lateral choroid plexus (cp) (1).The CSF in the developing brain is characterized by a high concentration of protein which originates mostly from blood plasma It has been suggested that a specific recognition mechanism for individual proteins is present at the blood–CSF barrier, during early stages of brain development but the molecular details are still unclear.
Results: In this study three genes: Secreted protein acidic and rich in cysteine (Sparc) Glycophorin A (GypA) and Glycophorin C (Gyp C) have been identified whose products are likely to target plasma proteins to cp cells. Cp epithelial cells from embryonic mice (E15) and from adult mice that were albumin or total plasma protein immunopositive have been analysed for these genes using quantitative and single cell PCR. It was shown that there was a significant concordance between plasma protein/albumin immunreactivity and expression of he putative transporter. Only in E15 cp epithelial cells SPARC and GypA was identified with immunohistochemistry with a subcellular distribution that was consistent with transport of albumin from blood to CSF. To investigate the putative albumin binding capacity of SPARC, glycophorin A and glycophorin C we applied a new sophisticated method to study protein-protein interactions, namely the in situ proximity ligation assay (in situ PLA). In situ PLA is a combination of immunological detection and PCR amplification and allows the visualization and quantification of protein-protein interactions on tissue sections or intact cells. Here we show the distribution of albumin bound to SPARC, glycophorin A or glycophorin C in the choroid plexuses of developing and adult mouse brains. Mechanisms for protein transfer across choroid plexus epithelial cells could operate will be proposed.

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ZO-1-VASP interaction controls tight junctional complex stability and function: Implication for age-related blood brain barrier permeability

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Blood brain barrier (BBB) permeability increases are usually due to structural changes in the brain endothelial junction complex itself. The magnitude of BBB hyperpermeability is closely associated with the degree of TJ complex changes which vary from complete loss of some TJ proteins (e.g. claudin-5 and occludin) in cases where is an uncontrolled increase in vascular permeability with robust plasma protein extravasation, leukocyte extravasation and development of vasogenic brain edema (BBB disruption), to morphologically fragmented or "difficult to detect" alterations in TJ proteins which cause small leaks that can persist over time and cause excessive build-up of fluid leading to brain dysfunction (BBB leakage). Insofar as the accumulating evidences define the morphological alteration and underlying mechanism of the TJ alteration in BBB breakdown, very little is know about type of alteration and mechanism of BBB leaking. Analysing the structural alteration of BBB in condition of "BBB leakage" associated with brain vascular dysfunction in aging mice, we found significant alteration in expression of (vasodilator-stimulated phosphoprotein (VASP) in condition of the increase permeability for small molecular size tracer Sodium Fluorescein and Inulin. As consequence of VASP downregulation there was diminished interaction between VASP-ZO-1, ZO-1 and actin as well as the ZO-1 and claudin-5. Rescuing VASP in this condition affect the stability of TJ complex and BBB permeability. Thus our further investigation led to identification the role of VASP and VASP-ZO-1 interaction in the organization and stability of the brain endothelial tight junction complex. Morphological and biochemical as well as FRET analysis indicated that VASP and ZO-1 are closely colocalized in brain endothelial TJ complex. Generating the series of the VASP mutants on EVH1 and PRP domain of VASP we found the regions in EVH1 and PRP domain responsible for direct interaction with PDZ and SH1 domain of ZO-1 respectively. Diminishing these interactions, affect the ZO-1-actin interaction as well as the ZO-1-claudin-5 interaction causing instability of TJ complex and increase permeability for Inulun. This study highlights the pattern and importance of ZO-1 and VASP interaction under basal conditions and in the aged BBB in regulating TJ complex stability and pinpoint VASP as a target for preventing and treating age-related BBB leakage.

Role of Notch-1 signaling in cocaine-mediated PDGF-BB expression: Implication for disruption of brain endothelial cells

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Background: Neuroinflammation associated with advanced HIV-1 infection is often exacerbated in cocaine-abusing, HIV-infected individuals. The underlying mechanisms are in part, attributable to disruption of the blood-brain barrier integrity modulated by cocaine. Platelet-derived growth factor-B (PDGF-B) chain, a potent mitogenic agent, has been implicated in a number of diverse pathologies underlying endothelial barrier dysfunction specifically in the central nervous system. The goal of this study was to determine whether there is a link between Notch signaling with PDGF-BB and dissect whether PDGF-BB is a novel immediate Notch target gene.
Methods: Cocaine-mediated induction of PDGF-BB involved the Notch signaling pathway in brain microvascular endothelial cells using Western blot and real time RT-PCR. CHIP assay was employed for validation of targeted expression of PDGF-BB induced by CSL. In vitro cell permeability and *in vivo* BBB permeability were used to confirm the role of Notch pathway in regulation of PDGF-B as a vascular permeant as evidenced by Evans blue and sodium fluorescence extravasation assays.
Results: In the present study, cocaine induced PDGF-B through the Notch signaling pathway in brain microvascular endothelial cells. Exposure of cells to the gamma secretase inhibitor-DAPT or silencing of Notch intracellular domain resulted in abrogation of cocaine-mediated induction of PDGF-B chain. Reciprocally, activation of the Notch 1 receptor by exposure of cells to the Notch ligand Jagged upregulated expression of PDGF-B chain. Furthermore, it was demonstrated that cocaine-mediated activation of Notch1 signaling leading to targeted expression of PDGF-BB involved activation of the major effector CSL. Functional implication of up-regulated PDGF-BB as a vascular permeant was confirmed *in vitro* in cell permeability assays. *In vivo* relevance of these findings was further corroborated in cocaine-treated mice that demonstrated increased permeability of the endothelial barrier. Specificity of Notch 1 signaling *in vivo* was validated in mice exposed to DAPT that failed to demonstrate barrier disruption following cocaine exposure.
Conclusions: This is the first evidence of involvement of Notch activation in cocaine-mediated regulation of PDGF-BB expression. Understanding how Notch-1 regulates PDGF-BB expression may provide insights into the development of potential therapeutic targets for neuroinflammation.

Role of STAT3 in the cochlear vascular barrier

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Signal transducers and activators of transcription 3 (STAT3) is a stress responsive transcription factor that relays signals from ligand-bound cytokine and growth factor receptors in the plasma membrane to the nucleus. Many of its target genes, such as VEGF, MnSOD, HIF-1α, and Survivin, are involved in the regulation of pro-survival and cellular proliferation functions. Through transcriptional regulation of VEGF, a prominent proangiogenic factor, STAT3 plays a major role in vascular paracellular permeability and angiogenesis under both normal and pathological conditions. VEGF, in turn, leads to increased STAT3 phosphorylation and activation through interaction with its receptor, VEGFR-2. In our studies on noise-induced stress responses in the inner ear, we observed that acoustic trauma increased STAT3 protein levels in the capillaries of the stria vascularis. Further examination revealed that noise exposure induced the phosphorylation and nuclear translocation of STAT3 in many cell types in the inner ear including marginal cells of the stria vascularis. Increased VEGF expression was also observed in this cell type following noise exposure. Using isolated stria vascularis capillaries and super resolution-structured illumination microscopy, VEGF treatment was found to increase the levels of phosphorylated STAT3 in capillary endothelial cells as well as the loss of beta-catenin from adherens junctions. That STAT3 is a key player in the cochlear blood-labyrinth-barrier signal transduction pathway is being explored through the use of the specific JAK/STAT3 inhibitorJSI-124.

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Sphingosine 1-phosphate receptor 5 mediates the immune quiescence of the human brain endothelial barrier

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The sphingosine 1-phosphate (S1P) receptor modulator FTY720P (Gilenya®) potently reduces relapse rate and lesion activity in the neuro-inflammatory disorder multiple sclerosis (MS). Although most of its efficacy has been shown to be related to immunosuppression through the induction of lymphopenia, it has been suggested that a number of its beneficial effects are related to altered endothelial and blood-brain barrier functionality. However, to date it remains unknown whether brain endothelial S1P receptors are involved in the maintenance of the function of the blood-brain barrier thereby mediating immune quiescence of the brain. Here we demonstrate that the brain endothelial receptor S1P5 largely contributes to the maintenance of brain endothelial barrier function. We show that activation of S1P5 on cultured human brain endothelial cells by a selective agonist elicits enhanced barrier integrity and reduced transendothelial migration of monocytes in vitro. These results were corroborated by genetically silencing of S1P5 in brain endothelial cells. Interestingly, functional studies with these cells revealed that S1P5 strongly contributes to brain endothelial cell barrier function and underlies the expression of specific blood-brain barrier endothelial characteristics such as tight junctions and permeability. In addition, S1P5 maintains the immunoquiescent state of brain endothelial cells with low expression levels of leukocyte adhesion molecules and inflammatory chemokines and cytokines through lowering the activation of the transcription factor NF-B. Our findings demonstrate that S1P5 in brain endothelial cells contributes for optimal barrier formation and maintenance of immune quiescence of the barrier endothelium.

The Matrix Reloaded: Perlecan Domain V and the Blood-Brain Barrier in Stroke and Alzheimer's Disease

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The role of the extracellular matrix in ischemic stroke and Alzheimer's disease (AD) is poorly understood. In stroke, it is typically thought to be degraded and a post-stroke marker of blood-brain barrier dysfunction. In AD, it is often thought to serve as a sink for extracellular accumulation of amyloid beta (Abeta), a key AD pathogen. However, perlecan domain V (DV), a proteolytic fragment of the vascular basement membrane, is persistently generated after stroke and is neuroprotective, enhances angiogenic brain repair, and inhibits chronic glial scar formation (a potential barrier to brain repair) when administered after transient middle cerebral artery occlusion in rodents. Furthermore, in AD, DV prevents Abeta mediated neurotoxicity. We now investigated whether DV could also be therapeutic in a permanent focal ischemia model in both young and aged mice and whether it might enhance neurorepair. In separate studies we investigated the potential of DV to decrease perivascular deposition of Abeta in a rodent AD model, and increase its transit across an endothelial cell monolayer in vitro. We demonstrate that DV was neuroprotective in both young and aged mice and that these animals had significantly improved post-stroke motor function measured by the cylinder and grid-walking tests. Additionally, DV enhanced several aspects of post-stroke neuronal regeneration including neurogenesis in the subventricular zone, migration, and neurorestoration in the peri-infarct region and ischemic core. *In vitro* analysis demonstrated that DV significantly increased neurogenesis, neuronal migration, and neurite sprouting. In AD studies, chronic DV treatment significantly decreased Abeta brain perivascular deposition in vivo and increased Abeta transit across an endothelial cell monolayer in vitro. Collectively, these results suggest that DV may represent a novel stroke and AD therapy.

The roles of β1 integrins in endothelial tight junction and microvessel permeability barrier integrity during focal cerebral ischemia

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Cerebral microvessels, their dependent neurons, and the other components of the "neurovascular unit" suffer a sequence of consistent and complex changes following focal cerebral ischemia. Endothelial cells and astrocyte end-feet adhere to components of the basal lamina by matrix adhesion receptors. These consist of β1 integrins for the endothelium, and the integrin α6/β4 and α/β-dystroglycan, predominantly expressed by astrocyte end-feet. During focal ischemia endothelial cell expression of the β1 integrins -α1, -α3, and -α6 decreases significantly within 2 hours of middle cerebral artery occlusion in the non-human primate, at a time when the permeability barrier opens and edema accumulates. This suggests the hypothesis that ligation of β1 integrins to the subtending basal lamina matrix is necessary for an intact permeability barrier. In vitro studies employing pure primary murine endothelial cells (and astrocytes) exactly mimic under normoxia and experimental ischemia the in vivo findings. Recently, we have shown that functional blockade of intact β1 integrin-matrix interactions, developed on confluent cerebral microvascular endothelial cells that express the inter-endothelial cell tight junction (TJ) proteins claudin-5, occludin, and ZO-1 after the cells have reached confluence, results in i) disorganization of all three TJ proteins, ii) decrease in their inter-endothelial cell expression, and iii) significant increase in permeability. The increase in permeability is confirmed by stereotaxic injection experiments in adult mice. No evident alterations in matrix integrity were noted. These results indicate that matrix-β1 integrin-TJ interactions are coordinated and that alterations seen in any one of the components of this complex suggest changes in the others. The implications of these findings for cerebral microvessel integrity and function, their contribution(s) to neuron function in the "neurovascular unit," signaling, and treatment approaches will be discussed.

Nrf2 Quantified in Endothelium of Core and Peri-Infarct Regions of Rat Brain Following Ischaemia-Reperfusion

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Activation of the redox sensitive transcription Nrf2 protects against ischaemia-reperfusion injury via upregulation of antioxidant defence genes. We have quantified Nrf2 content in brain tissue and endothelium in rats subjected to stroke using a novel immunohistochemical technique. Rats were subjected to 70 min middle cerebral artery occlusion (MCAo) and reperfusion for either 24h or 72h. Coronal 10 -m brain sections were incubated with an anti-Nrf2 antibody and HRP conjugated secondary. RECA-1 and GFAP were used to define the endothelium and astrocytes by immunofluorescence. When sections were reacted with DAB in the presence of H2O2, the initial rates of DAB polymer formation were directly proportional to the concentration of Nrf2. Image processing was used to determine the distribution of Nrf2 in nuclear, cytoplasmic and endothelial compartments in stroke regions and the unaffected contralateral hemisphere. Increased nuclear to cytoplasmic distribution of Nrf2 was observed after 24h reperfusion, with increased levels in the periphery and core of stroke affected regions versus contralateral regions. Nrf2 content decreased after 72h reperfusion and similar changes were observed in endothelial cells after 24h and 72h reperfusion. Our findings provide the first quantitative measurements of Nrf2 content in rat brains subjected to ischaemia-reperfusion injury. The increased Nrf2 content in the infarct core and periphery and in brain microvessel endothelium at 24h compared to 72h, highlights important time-dependent changes in Nrf2 distribution in brain tissue after stroke.

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Attenuation of High Glucose-Induced Oxidative Stress and Apoptosis in Brain Pericytes by a Mitochondrial Carbonic Anhydrase Inhibition

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Diabetes mellitus has strongly been associated with overproduction of reactive oxygen species (ROS) during electron transport reactions. Overproduction of mitochondrial ROS results in oxidative stress which is augmented during diabetes in insulin-insensitive tissues such as brain. Pericytes (PC) in the microvasculature of brain are especially susceptible to oxidative stress and subsequent apoptosis. Mitochondrial carbonic anhydrases CA VA and VB (mCAs) regulate oxidative metabolism of glucose and thus play an important role in ROS generation and oxidative stress. We have recently shown that inhibition of mCAs reduces diabetes-induced oxidative stress in the mouse brain and rescues cerebral PC loss. Here we tested the hypothesis that pharmacological inhibition of mCAs attenuates high glucose-induced intracellular oxidative stress and apoptosis of cerebral PC in culture.

We isolated conditionally immortal cerebral PC (imPC) from transgenic Immorto mouse. To assess morphological and physiological relevance of imPC to primary cerebral PC, these cells were analyzed for primary PC markers and transendothelial electrical resistance (TEER). The presence of mCAs was ascertained by RT-PCR and Western blotting. The cells were grown in normal glucose (5.7 mM) and high glucose (40.7 mM) for 6 days. The measures of oxidative stress and of oxidative damage were reduced glutathione (GSH), and 4-hydroxy-trans-2-nonenal (HNE), respectively. Apoptosis was assessed by TUNEL. For pharmacological inhibition of mCAs, ethoxyzolamide (ETZ) or topiramate (TOP) was added to the culture medium from day one of the experiment.

The imPC expressed PDGFR-β, NG2, CD13, and α-SMA markers. PECAM-1, the endothelial cell marker was not expressed. The cells were successfully passaged and maintained in culture for several months without loss of these markers. The PC caused high TEER and low permeability of endothelial cells comparable to primary PC. Six days exposure to high glucose resulted in a significant decrease in GSH, and significant increase in HNE and % apoptotic cells. Treatment of imPC with both ETZ and TOP significantly reduced high glucose-induced oxidative stress and apoptosis.

These results provide the first evidence that high glucose induces oxidative stress and apoptosis in mouse brain imPC. Furthermore, pharmacologic inhibition of mCAs attenuates oxidative stress and imPC apoptosis caused by high glucose.

The role of conserved cysteines in the *cis*- and *trans*- homooligomerization of occludin under oxidative stress

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Brain capillary endothelial cells form the BBB. The paracellular cleft between the endothelial cells is sealed by tight junctions (TJ). TJ maintain cell polarity and limit paracellular permeation. Although being the first identified transmembrane protein of the TJ, the function of occludin remains an open question. It is assumed to be a regulatory protein due to the existence of multiple phosphorylation sites. Recent findings give hints that occludin may act as a molecular target for the TJ organization under pathological conditions (e.g. oxidative stress). It has already been shown that occludin undergoes changes in its cellular localization and oligomerization state under oxidative stress (Walter et al., 2009).
So far, the redox-dependent occludin alterations where thought to be based on a cysteine reaction on position 409 in the human sequence. Furthermore, there is the assumption that the cysteines of the transmembranal domains and the 2nd extracellular loop (ECL2) (McCaffrey et al., 2008) may play a role in the oligomerization of occludin in *cis* (association of molecules within one plasma membrane) and *trans* (association of molecules located within membranes of adjacent cells). The results show that transmembranal- and ECL2 localized cysteines can influence the membrane localization (C216A, C237A) as well as the *trans*- (C76A, C148A, C216A, C237A) and *cis*-interactions (C82A, C216A, C237A). Redox sensitivity was shown for the residues C82 (transmembrane domain 1), C216 and C237 (both ECL2). We assume that the ECL2 shows a redox depending intramolecular binding of the residues 216 and 237. This is first evidence that the ECL2 contains a loop within itself which plays an important role for the *cis* and *trans* interactions of occludin.
In summary the results support the concept, that redox changes regulates the structure and function of occludin and thereby influences the structure and function of the TJs in general.

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What does the blood-brain barrier protect the brain from?

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The Blood-brain barrier (BBB) is a complex structural and functional barrier controlling and maintaining a distinct extracellular environment within the brain. In the last decade experimental data has been accumulating revealing the effects of BBB dysfunction on brain functions. Here I will summarize physiological experiments performed in rodents in-vivo and in-vitro in which the brain was exposed to "blood-like" conditions. Our data indicate that proper function of the BBB is crucial for the maintenance of normal neuronal excitability, synaptic transmission and synaptic plasticity. In addition, the protection of the BBB from serum proteins is a key signaling mechanism to control glia functions including brain immune response, and normal vascular response to neuronal activation. Finally, a proper BBB function is a key to pharmacotherapeutics of both peripheral and centrally-acting drugs: BBB opening may allow the delivery of peripherally acting drugs into the central nervous system, causing unwanted neurological signs and symptoms. BBB dysfunction may also alter the effect of brain-permeable drugs, and underlies pharmacoresistance. The direct clinical implications of these new insights into BBB function and dysfunction highlight the importance of standard imaging protocols for the reliable diagnosis of BBB dysfunction.

Vascular homeostasis in malignant brain tumors

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In the adult brain, vascular integrity is important for brain homeostasis. In pathological conditions such as brain tumor growth, vascular integrity is significantly disturbed. Our lab focusses on tumor derived secreted growth factors that interact with the vascular endothelium (such as VEGF´s and Angiopoietins) and thereby regulate vascular functions.
In a previous study in human glioblastoma specimens, we have shown that the antagonistic ligand of the Tie-2 receptor tyrosine kinase, Angiopoietin-2 (Ang-2), is selectively up-regulated in tumor endothelial cells but remains at low levels in vessels within the normal brain. In contrast, the expression of the agonistic ligand Angiopoietin-1 (Ang-1) was not altered to a significant extent. We therefore concluded that selective up-regulation of agonistic Ang-2 would lead to downregulation of Tie-2 phosphorylation in situ. We have recently confirmed this conclusion in a transgenic mouse model with inducible, endothelial cell-specific expression of Ang-2 in brain tumors. In order to investigate the consequences of Ang-2 up-regulation and concomitant blockage of Tie-2 phosphorylation, we examined the tumor vasculature for endothelial cell integrity and pericyte detachment. Our findings are in line with the hypothesis that Ang-2 leads to detabilized tumor vessels. In human brain tumor specimens, Ang-2 protein expression was detected by a novel antibody and was found to be confined to endothelial cells. Further, Ang-2 expression levels correlated with both WHO grade and the number of infiltrating monocytes/macrophages. In order to investigate whether Ang-2 regulates monocyte/macrophage influx in gliomas we made use of Ang-2/Tie-1 TTA transgenic mice. Under physiological conditions, (e.g. without any pathological stimulus), CD11b+ cells increased in almost all tissues of transgenic mice in a time-dependent manner upon prolonged expression of Ang-2. This influx of CD11b+ myeloid cells was dependent on ß2-integrin function. In transplanted gliomas, the number of infiltrating cells further increased significantly. Our findings therefore identify endothelial derived Ang-2 as a novel regulator of myeloid cell infiltration in gliomas in vivo.

The Side Population phenotype in human Glioblastoma is exclusively stroma-derived and its efflux properties are unaffected by anti-VEGF treatment

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Progression of glioblastoma is proposed to be triggered by a cancer stem cell population, postulated to be responsible for tumor recurrence due to their resistance to radio- and chemotherapy. Resistance mechanisms may involve ATP-binding cassette (ABC) transporters on the cell membrane, which are responsible for drug efflux from the cell and may therefore represent putative cancer stem cell (CSC) markers. We investigated the presence of the Side Population (SP) phenotype, which is recognized by increased efflux of the Hoechst dye through ABC transporters, in human glioma biopsies as well as in intra-cranial xenograft models derived from human biopsy spheroids and stem-like glioma cultures. We used a GFP expressing immunodeficient mouse model, enabling to separate tumor cells from host stromal cells, thereby allowing to clearly identify the cellular origin of the SP cells. Interestingly we find that SP cells in human gliomas is uniquely stroma-derived, thus indicating that the SP phenotype is not a valid marker for glioma CSCs. Indeed the SP population present in glioma tissue is composed of endothelial and astrocytic cells, whereas neither stromal nor tumor-derived stem/progenitor populations in the adult brain possess efflux properties. We further determined the effect of anti-angiogenic treatment by bevacizumab on the efflux properties of stroma-derived endothelial cells in order to address the question whether normalization of the vasculature induced by anti-VEGF agents constitutes an advantage or an impediment for drug delivery in the brain. The results on stromal-derived as well as tumor-derived endothelial cells will be discussed.

Role of extracellular RNA in regulating adhesion and transmigration of tumor cells at the blood-brain barrier

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Extracellular RNA (eRNA), released from cells under injury or pathological conditions, has been shown to act as prothrombotic factor and further to induce vascular endothelial growth factor (VEGF)-dependent hyperpermeability of the blood-brain barrier (BBB) in vivo and in vitro. RNase1 pretreatment reduced all these functional activities of eRNA. The aim of this study was to investigate the role of eRNA regarding the adhesion and transmigration of tumor cells across the BBB.
Human glioblastoma tumor tissue contained high amounts of eRNA as was shown by immunhistochemistry. Tumor cell lines like the human colon carcinoma cell line HT29 or the fibrosarcoma cell line HT1080 released higher amounts of eRNA into their cell supernatants in comparison to non-tumor cells, which was even induced under hypoxia. Involvement of eRNA in tumor growth was confirmed in vivo after injecting HT29 subcutaniously into NUDE mice, whereby RNase but not DNase treatment significantly decreased tumor volume and weight. Using an in vitro model of the BBB, eRNA induced the adhesion and transmigration of HT1080 or THP1 cells, a monocytic cell line, to and across monolayers of human cerebral microvascular endothelial cells (HCMEC/D3). Both, eRNA-induced adhesion and transmigration of tumor cells, were dependent on the activation of the Vascular Endothelial Growth Factor (VEGF)/VEGF receptor system. Additionally, eRNA induced a high release of TNF-α from monocytes and macrophages, which involved activation of TNF-α-converting enzyme (TACE), a membrane-bound disintegrin metalloproteinase also known as ADAM17. Specific inhibitors of TACE inhibited RNA-induced release of TNF-α completely but not of IL-6. Accordingly, supernatants from tumor cells induced the release of TNF-α from macrophages, which was abolished by RNase. Furthermore, supernatants derived from RNA-treated macrophages enhanced the adhesion of tumor cells to HCMEC/D3, which was abolished by neutralizing antibodies against TNF-α. RNA-induced TNF-α release involved signaling via the NF-κB pathway and further activation of p38 MAPkinase. Staining of glioblastoma tumor tissue revealed that areas containing high concentrations of eRNA correlated to sites where high concentrations of macrophages and TNF-α were detectable. Results indicate that eRNA released from tumor cells may induce their adhesion and transmigration across the BBB directly but also indirectly by inducing the release of TNF-α from infiltrated monocytes/macrophages.

Neuroinflammation Inhibits Abeta Efflux: Implications for Alzheimer's Disease

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Amyloid beta peptide (ABP) is thought to play a causal role in Alzheimer's disease (AD). Although ABP is elevated in AD patients, the elevation is caused in the vast majority not by overexpression but by clearance impaired either by enzymatic processing or blood-brain barrier (BBB) efflux. The two efflux transporters for ABP at the BBB are p-glycoprotein (Pgp) and low density lipoprotein receptor-related protein-1 (LRP), both of which are known to be impaired in AD. We and others have shown that both LRP and Pgp function are impaired when the innate immune system is activated by injections of lipopolysaccharide (LPS). Bulk flow is also reduced with LPS treatment as is peripheral clearance of amyloid beta by kidney and liver. We have found that the LPS-induced impairment in efflux of radioactive ABP is blocked by indomethacin, an inhibitor of prostaglandin production, and by N-acetylcysteine (NAC), a molecule with antioxidant and anti-inflammatory properties. NAC effectively reverses the impaired efflux attributed to LRP but not that attributed to Pgp. Capillary depletion indicates that the impairment in LRP function is in its intracellular trafficking and LPS induces a redistribution of LRP in brain endothelial cells grown in monolayers. LPS induces a reduction in protein levels of LRP in brain endothelial cells but increases levels of LRP in brain pericytes. Thus, we have found 5 mechanisms by which LPS would act to increase brain levels of abeta: 1) increased presentation of circulating ABP (because of decreased clearance by liver and kidney); 2) decreased efflux by Pgp; 3) decreased efflux by LRP; 4) decreased efflux by inhibition of bulk flow; 5) increased brain retention because of increased uptake by pericytes. Overall, these suggest that inflammation could play a key role in increased brain levels of ABP in large part through actions at the BBB.

Interactions of HIV-1 with Amyloid Beta Peptide at the Blood-Brain Barrier Level

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Due to the success of antiretroviral therapy there is a sharp increase in age of HIV-1 infected patients. HIV-1 brains are characterized by increased deposition of amyloid in perivascular space, indicating the importance of brain microvessels and the blood-brain barrier in amyloid accumulation. In the current study, we evaluated the mechanisms of HIV-1-induced amyloid beta accumulation in brain endothelial cells and its transendothelial passage. Exposure to HIV-1 resulted in a markedly increased amyloid beta levels in hCMEC/D3 cells. Both silencing of caveolin-1 (cav-1) and disruption of lipid rafts protected against these effects. Exposure to HIV-1 activated caveolae-associated Ras and p38. While inhibition of Ras effectively protected against HIV-1-induced accumulation of amyloid, blocking of p38 did not have such an effect. We also evaluated the role of caveolae in HIV-1-induced upregulation of the receptor for advanced glycation end products (RAGE), which regulates amyloid transfer from the blood stream into the central nervous system. HIV-1-induced RAGE expression was prevented by infecting hCMEC/D3 cells with cav-1 specific shRNA lentiviral particles or by Ras inhibition. Using transgenic mice that express a chimeric mouse/human amyloid precursor protein and a mutant human presenilin 1, we next demonstrated that cerebrovascular toxicity of HIV specific protein Tat is enhanced in mice with amyloid deposits in the brain. Indeed, exposure to Tat increased permeability across cerebral capillaries, enhanced disruption of ZO-1 tight junction protein, and elevated brain expression of matrix metalloproteinase-9 in transgenic mice as compared to age-matched littermate controls. These changes were associated with increased leukocyte attachment and their transcapillary migration.

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Genetic evidence for LRP1 and PrP^c mediated amyloid-β transcytosis across the blood-brain barrier

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According to the 'amyloid hypothesis', the amyloid-β (Aβ) peptide is the toxic intermediate driving Alzheimer's Disease (AD) pathogenesis. The blood-brain barrier (BBB) facilitates Aβ exchange between blood and brain, which is likely to impinge on Aβ brain homeostasis and, therefore, its neurotoxicity. Recent evidence suggests that the low density lipoprotein receptor-related protein 1 (LRP1) transcytoses Aβ out of the brain. To provide genetic evidence for LRP1-mediated transcytosis of Aβ across the BBB we analysed Aβ transcytosis across primary mouse brain capillary endothelial cells (pMBCECs) derived from wild-type and LRP1 knock-in mice. We show that pMBCECs *in vitro* express functionally active LRP1. Moreover, we demonstrate that LRP1 mediates transcytosis of [^{125}I]-Aβ_{1-40} across pMBCECs in both directions, whereas no role for LRP1-mediated Aβ degradation was detected. Analysis of [^{125}I]-Aβ_{1-40} transport across pMBCECs generated from mice harbouring a knock-in mutation in the NPxYxxL endocytosis/sorting domain of endogenous LRP1 revealed a reduced Aβ clearance from brain-to-blood and blood-to-brain compared to wild-type derived pMBCECs. For the first time, we present genetic evidence that LRP1 modulates the pathogenic actions of soluble Aβ in the brain by clearing Aβ across the BBB. In addition, we found that the cellular prion protein (PrP^c), a putative receptor implicated in mediating Aβ neurotoxicity in Alzheimer's disease (AD) which has been shown to be co-internalized with LRP1, participates in Aβ transcytosis across the BBB. Using our *in vitro* BBB model, Aβ transcytosis was reduced by genetic knock out of PrP^c or after addition of a competing PrP^c specific antibody. Furthermore, we provide evidence that PrP^c is expressed in endothelial cells and, that monomeric Aβ binds to PrP^c. These observations provide new mechanistic insights into the role of LRP1 and PrP^c in AD.

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APOE, Agrin, Microvascular Injury and Blood Brain Barrier Compromise in Sporadic (Late Onset) Alzheimer's Disease

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The late onset sporadic form of Alzheimer's disease (90%) has been linked to the APOE4 gene on chromosome 19, which codes for the high-density lipoprotein ApoE4. Such patients typically exhibit symptoms of cognitive impairment later in life; have a more slowly progressive clinical course and a variable degree of brain AD pathology. Despite the unequivocal association between APOE4 and late-onset sporadic AD, the mechanism(s) through which APOE4 contributes to the pathogenesis of sporadic AD remain(s) elusive.

Numerous brain imaging studies have documented a preferential decrease in cerebral blood flow to brain areas affected by AD, as well as an increase in small vessel disease in Alzheimer patients. Various components of the fragmented vascular basement membrane are found within senile (neuritic) plaques raising the question of whether plaque formation and microvascular pathology are somehow closely linked.

Previous studies by our group and others have documented that agrin, the major heparan-sulfate proteoglycan component of the cerebral capillary basement membrane, becomes fragmented in sporadic AD compromising microvascular structural integrity. We have also demonstrated that this structural damage is greater in AD patients with the APOE4 genotype and correlates with the appearance of the serum derived protein prothrombin in the brain, presumably due to a defective blood-brain barrier. To examine the relationship of agrin, the BBB, and Alzheimer's-related pathologies, we generated mice in which the gene (Agrn) was specifically deleted from endothelial cells or neurons using gene-targeting, or overexpressed using a genomic transgene construct. These mice were combined with a transgenic model of AD. We found that in mice lacking endothelial expression of Agrn, the BBB was intact, but aquaporin4 levels were reduced, indicating the loss of agrin is affecting other BBB-associated components. This change in Agrn resulted in an increase in A-beta in the brain, whereas elimination of Agrn from neurons did not change A-beta levels, and overexpression of Agrn decreased A-beta deposition. These results indicate that agrin is important for maintaining BBB composition, and that changes in Agrn expression influence A-beta homeostasis in mouse models of Alzheimer's disease.

Pharmacologic and epigenetic manipulations, related to preserving the neurovascular unit and BBB, clearly represent an exciting new approach for reducing the onset and progression of dementia in sporadic AD patients.

Pathophysiology of Lysosomal Storage diseases as model for neurodegenerative diseases

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According to World Health Organization, 10 % of the world population will suffer or die because of a disease related to the Central Nervous System (CNS), furthermore, it is widely known that a considerable number of so called "CNS drugs" actually do not reach the brain due to the high selectivity and efficiency of the blood brain barrier (BBB). For all these reasons, the availability of a model disease to be used to study pathophysiology of the brain disorder and to test new therapeutic approaches might be desired.
Lysosomal Storage Diseases (LSDs), a small group of about 60 disease, may satisfy this desire. They are monogenic, one gene defect is causing one disease, the stored material is well known, the secondary cascade events caused by the storage can be studied and reliable animal models mimicking the human disease are available. Furthermore, up to 70% of the patients are affected by major CNS involvement.
These characteristics and the discovery of the involvement of the lysosome in important cellular pathways did allow cell biologist to elevate the lysosome to the rank of relevant crossway of the cellular metabolism.
In fact, it has been shown that the lysosome is interacting with mitochondria, Golgi apparatus and reticular endoplasm as well as systems for the intracellular homeostasis . Lysosomal alterations and storage materials are responsible of profound effects on the cell metabolism: accumulation of secondary materials, dysfunction of mitochondria respiration and cellular distress and activation of cytokines and inflammatory response are the major cause of cell death.
On a clinical side, cellular distress and death are responsible of the chronic and progressive neurodegeneration, resulting in profound alteration of the neurological brain structure both at microscopic and macroscopic levels. A wide variety of neurological signs and symptoms will occur and will lead patients to severe impairment and eventual death. This same pattern is in common with socially relevant diseases such as Alzheimer and Parkinson diseases where lysosomes are also involved in the defective degradation of stored material.

Biological markers of severity in lysosomal storage disease

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CNS early onset disorders in Sanfilippo syndrome are responsible for cognitive and behavioural manifestations leading to progressive mental retardation and neurodegeneration. Although pathology is ubiquitous, manifestations outside the CNS are mild. Disorders have a unique cause, the deficiency of a lysosomal enzyme, which interrupts the degradation of heparan sulfate (HS) in lysosomes and causes permanent release of HS oligosaccharides in the environment through lysosomal exocytosis. HS fragments bound on extracellular matrix (ECM) induce multiple deleterious consequences including: i) constitutive activation of integrins and stabilization of microtubules, which affect Golgi organisation, cell division, cell polarization, cell migration and neuritogenesis; ii) activation of TLR4-MyD88 in microglia, causing chronic neuroinflammation; iii) modified expression of genes involved in ECM protein turnover.
Our studies performed in animal models of the disease led to the definition of biomarkers measurable in brain tissue that are indicative of disease severity. Preclinical studies performed in 35 affected dogs demonstrated the safety of a gene therapy procedure based on the deposition of AAV vectors coding for the missing enzyme in the brain and provided evidence for the correction of biomarkers in tissue. Phase I/II clinical studies are currently ongoing to assess tolerance of this treatment in children. Efficacy assessment will rely on the detection of disease biomarkers in patient's CSF. Material collected during dog studies is currently used to specify molecules in the CSF, the detection of which varies with disease progression and treatment. They include detection of therapeutic enzyme, HS oligosaccharides, and unbiased quantitative proteomic studies.

microRNAs control brain endothelial cell barrier function and immune quiescence, implications for MS

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In normal physiology the blood-brain barrier (BBB) tightly regulates brain homeostasis. Perturbations of BBB function, including the loss of brain endothelial cell barrier integrity and immune activation are hallmarks of multiple sclerosis. Therefore, understanding of the BBB in health and disease may lead to novel approaches for MS treatment.
Using a combined genetic and bioinformatics approach, we uncovered a novel mechanism which regulates different aspects of the BBB, including barrier formation and immune quiescence, i.e. through microRNAs. microRNAs are recently discovered endogenous, small, noncoding RNAs which regulate the production of about 30% of human proteins and have been shown to be involved in cell biology and pathology. Using a genomics approach, we have identified a microRNA (miR-125a-5p) which targets the activity of the transcription factor myc-associated zinc finger protein and plays a major role in the formation of a tight brain endothelial cell barrier and the paracellular trafficking of immune cells. Interestingly, lower levels of miR-125a-5p were associated with the inflamed BBB in vitro and in brain endothelial cells obtained form MS patients by laser capture. Most importantly, our recent analyses in brain capillaries which were isolated from MS patients have revealed that a large panel of BBB stabilizing microRNAs is significantly reduced in MS lesions.
We conclude that therapeutic application of microRNAs (such as miR-125a-5p) potentially could re-establish normal functioning of the BBB to prevent inflammation in multiple sclerosis.

Pericyte-regulated functions of the BBB

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The CNS endothelial cells possess several specific features (e.g. closed endothelial junctions, low rate of transcytosis) collectively named the blood-brain barrier (BBB), that regulate the passage of proteins and other bioactive molecules from blood to the brain parenchyma. The abluminal side of the CNS endothelium is covered by pericytes and astrocyte end-feet which together form the neurovascular unit. The relative contribution of different cellular components of the neurovascular unit to the formation and regulation of the BBB is largely unknown. Pericytes have been suggested as being important regulators of the BBB, however, the in vivo evidence has been lacking. By analyzing the integrity of the BBB/neurovascular unit of viable pericyte-deficient mouse models we demonstrate for the first time in vivo the importance of pericytes for the integrity of the blood-brain barrier and organization of the astrocyte end-feet. In the absence of pericytes the brain endothelium shows increased uptake of intravenously administered tracers and subsequent release into the brain parenchyma. I will also present data which indicates that in addition to the deregulated transcytosis from blood to brain, the brain to blood transport route is altered in pericyte-deficient mice.

The Blood-Brain Barrier: Checkpoint Charlie for immune cell entry into the CNS

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The central nervous system (CNS) is an immunologically privileged site to which access of circulating immune cells is tightly controlled by the endothelial blood-brain barrier (BBB) localized in CNS microvessels and the epithelial blood-cerebrospinal fluid barrier (BCSFB) within the choroid plexus. Due to the specialized structure of the CNS barriers, immune cell entry into the CNS parenchyma involves two differently regulated steps: migration of immune cells across the BBB or BCSFB into the cerebrospinal fluid (CSF) drained spaces of the CNS, followed by progression across the glia limitans into the CNS parenchyma. With a focus on multiple sclerosis and stroke and their animal models I will summarize the distinct molecular mechanisms reqired for immune cell migration across the different CNS barriers.

Leukocyte-endothelial cell cross talk at the blood brain barrier: the good and bad side of a two way system

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Molecular inflammatory processes occurring in the periphery are not 'silent' but can be perceived by the brain, the crucial discriminatory player being the blood brain barrier (BBB). BBB (composed of more than solely endothelial cells) represents the interface between the circulation and the brain and plays a fundamental role mediating cross-talk between the two compartments. Consequently the interaction between circulating leukocytes and the BBB endothelium, and their subsequent transmigration is a critical step in the pathogenesis of many CNS diseases. We have previously shown Annexin A1 (ANXA1), a mediator of glucocorticoid action in the peripheral system, to be a fundamental regulator of BBB tightness (1), restricting paracellular permeability. Here we show that Anxa1 null mice present a higher density of Iba1 positive cells (monocytes/microglia) in the substantia nigra compared with wild-types, and that peripheral administration of LPS (3mg/kg body weight) induces a further increase in Iba1 positive cell density that does not resolve in the AnxA1 null mice. Moreover, the morphology of Iba1 positive cells in young AnxA1 null mice was highly reminiscent of cells in aged individuals, correlating with published data indicating a decline in ANXA1 expression as a marker of aging. To study the involvement of this endogenous "defensin" we analysed the interactions under shear stress of human peripheral blood mononuclear cells with a human brain immortalised microvascular cell line (2) stably infected with shRNA for ANXA1. Significantly greater numbers of PBMCs adhered to endothelial monolayers lacking ANXA1 under inflammatory conditions. We present here evidence that the lack of endogenous factors that occurs with normal ageing, such as the anti-inflammatory molecule ANXA1, may lead to inappropriately raised BBB permeability and entry of leukocytes into the brain, features which can render individuals prone to neurodegenerative disease.

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T cell extravasation across the Blood-brain barrier endothelium: Differential contribution of ICAM-1, ICAM-2, VCAM-1 and ALCAM to T cell adhesion, crawling and diapedesis

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Extravasation of T cells from the vasculature to the site of inflammation is a tightly regulated multi step process involving rolling, arrest, crawling and – finally - diapedesis across the endothelium. Although the blood brain barrier (BBB) is a highly specialized endothelium protecting the central nervous system (CNS) from harmful substances in the blood stream through an elaborate network of tight junctions, high numbers of encephalitogenic T cells extravasate across the BBB in the pathogenesis of multiple sclerosis. Therapeutic targeting of immune cell entry into the CNS has proven to be beneficial for treatment of multiple sclerosis patients. Unfortunately, the current treatment with the humanized anti-alpha4-integrin antibody natalizumab is associated with the rare risk to develop progressive multifocal leukoencephalopathy. Thus, alternative and safer targets involved in T cell trafficking into the CNS need to be identified. We focus on defining the individual roles of endothelial cell adhesion molecules (CAMs) in mediating T cell extravasation across the BBB. Using live cell imaging of T cell interaction with an in vitro mouse BBB model we delineated a sequential involvement of endothelial ICAM-1 and VCAM-1 in mediating shear resistant T cell arrest followed by endothelial ICAM-1 and ICAM-2 in mediating T cell crawling to sites permissive for diapedesis across BBB endothelium. Recently, endothelial ALCAM was described of being essentially involved in the extravasation of CD4+ T cells across the BBB. Therefore, we used our experimental expertise in studying the dynamic interaction of T cells with the BBB to analyse the role of endothelial ALCAM for the extravasation of encephalitogenic CD4+ T cells across the BBB.

Mixed Cerebrovascular Disease: Role for Hypertension in Cerebral Amyloid Angiopathy, Microhemorrhages and Neuroinflammation

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There is increasing recognition that cerebral vascular dysfunction plays a critical role in aging and neurodegenerative diseases, including AD where approximately 80-95% of the cases have cerebrovascular pathology. Furthermore, age-related cerebrovascular dysfunction contributes to ischemic stroke, intracerebral hemorrhages, and microbleeds. Apart from age, the greatest risk factor for cerebrovascular dysfunction is hypertension, and hypertension in midlife increases the risk of dementia in the elderly. Hypertension commonly causes cerebral small vessel disease (SVD) in the white matter, which increases the risk of stroke and dementia. The accumulation of A-40 in the cerebrovascular system, cerebral amyloid angiopathy (CAA), is a significant risk factor for intracerebral hemorrhage (ICH). We have developed several mouse models that combine hypertension protocols with amyloid precursor protein (APP) transgenic mice (Tg2576), which accumulate significant CAA-Type 2 in the large cerebral vessels and the meninges by 18 months of age. To induce hypertension in the mouse models, we used chronic administration of angiotensin II by subcutaneously implanted Alzet mini-osmotic pumps. Blood pressure was measured daily in conscious mice using a non-invasive tail-cuff blood pressure monitoring system. Clinical signs of stroke were measured using contralateral forelimb extension, circling behavior and other tests for motor dysfunction. The slow pressor model of hypertension was induced by chronic angiotensin II administration in mice for 28 days. There were no clinical signs of stroke in non-Tg littermates or Tg2576 mice, even though mean blood pressure values rose to greater than 150 mmHg by the end of the experiment. However, both non-Tg and Tg2576 mice displayed increased neuroinflammation in response to the hypertension as observed by activated phenotypes for microglia and astrocytes when compared to the PBS control mice. Hypertensive Tg2576 mice developed more CAA and a trend toward greater amyloid plaque load than PBS-treated Tg2576 mice. Finally, there was a significant increase in cerebral A- by ELISA in the hypertensive Tg2576 mice relative to the PBS group. Thus, even a short period of hypertension in Tg2576 mice can increase neuroinflammation, CAA, microhemorrhages, and parenchymal A-. Further studies may provide insights into the mechanisms of hypertension-induced changes in the cerebral vascular system that initiated the increase in CAA.

Inhibition of poly(ADP-ribose) polymerase (PARP)-1 protects blood brain barrier (BBB) in HIV CNS infection

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Despite immune recovery in individuals on combination antiretroviral therapy, the frequency of HIV associated neurocognitive disorders (HAND) remains high. HIV-associated neurodegeneration is driven by chronic inflammatory responses in the brain secondary to a low level of HIV replication in CNS macrophages/microglia and injury to the BBB mediated by pro-inflammatory factors in blood and migration of leukocytes across the BBB. Although the modulatory effects of PARP inhibitors on immune cells have been studied to some extent, nothing is known about their effects in the setting of HIV CNS infection. We investigated whether PARP inhibition will attenuate BBB injury caused by HIV-1 via effects on monocytes, brain endothelium, and activated HIV-1 infected macrophages. We found increased expression of PARP in brain endothelium and macrophages in human brains with HIV encephalitis. PARP suppression in primary human brain microvascular endothelial cells (BMVEC) improved BBB integrity, augmented expression of tight junction proteins and prevented barrier disruption caused by inflammation. PARP inhibition in BMVEC diminished monocyte adhesion/migration across a BBB model, downregulated adhesion molecules and decreased activity of RhoA/Rac1 (controlling BBB integrity and monocyte migration across the BBB). PARP inhibitors down regulated inflammatory genes increased by TNF-α in BMVEC. In monocytes, PARP inhibitors down regulated the active form of β-integrin that paralleled RhoA/Rac1 suppression. PARP inhibitors decreased expression of pro-inflammatory molecules and diminished HIV replication in macrophages. In vivo treatment with a PARP inhibitor decreased enhanced BBB permeability in mice with systemic inflammation. These results point to the relevance of PARP suppression in protection of the BBB in the setting of HIV-1 infection.

The involvement of the blood-CSF barrier in the african trypanosomiasis

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At the turn of the 19th century, trypanosomes were identified as the causative agent of sleeping sickness and their presence within the cerebrospinal fluid of late stage sleeping sickness patients was described. However, no definitive proof of how the parasites reach the brain has been presented so far. Analyzing electron micrographs prepared from rodent brains more than 20 days after infection, we present here conclusive evidence that the parasites first enter the brain via the choroid plexus. We never observed parasites within the neural parenchyma beyond the brain capillaries, but found them first within the fenestrated capillaries of the choroid plexus, from where they penetrate the endothelial cells, the stroma and the epithelial cell layer to reach the ventricular system. We also show that brain infection depends on the formation of long slender trypanosomes and that the cerebrospinal fluid as well as the stroma of the choroid plexus is a hostile environment for the survival of trypanosomes, which enter the pial cell layer (including the Virchow-Robin space) via the subarachnoid space to escape degradation. Our data suggest that trypanosomes do not intend to colonize the brain but reside near or within the glia limitans, from where they can re-populate blood vessels and disrupt the sleep wake cycles.

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CD147 is the receptor for pilus-mediated adhesion of meningococci to brain vascular endothelium

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Neisseria meningitidis (or meningococcus) causes human meningitis epidemics and rapidly progressing fatal shock worldwide. In particular, adhesion to brain vascular endothelium enables bacteria to cross the blood-brain barrier and to invade meninges. We recently demonstrated that pilus interaction with the host G-protein-coupled β2-adrenergic receptor (β2AR) is essential to promote signalling events following bacterial adhesion. However, β2AR–depleted endothelial cells still support initial attachment of meningococci by interacting with another as yet unidentified receptor.
Now we identified CD147 (also called EMMPRIN or Basigin), a member of the immunoglobulin superfamily, as a critical host receptor for the initial adhesion of *N. meningitidis* to human brain endothelial cells. CD147 depletion, soluble CD147 and antibodies targeting CD147 inhibited the primary attachment of *N. meningitidis* to human brain endothelial cells. Piliated meningococci specifically bound to immobilised CD147 and pilus components involved were identified. *Ex-vivo* infection of human brain sections revealed that meningococci specifically adhere to CD147-expressing endothelial cells, this adhesion being inhibited by anti-CD147 antibodies. These findings represent a breakthrough for understanding initial events leading to meningeal invasion and provide new targets for treatment and prevention of meningococcal infection.

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A two-signal model for the activation of the blood-CSF barrier for immune cells trafficking

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The healthy brain is an immune privileged site, shielded by barriers from circulating immune cells. Nevertheless, numerous studies have suggested that a continuing dialogue between the brain and circulating immune cells is needed to maintain life-long brain plasticity, including neurogenesis, cognitive ability, and resilience to stress. Since the blood-cerebrospinal fluid barrier (BCSFB), at the brain's borders, is constantly exposed to circulating immune cells, we hypothesized that at this junction, the choroid plexus (CP) epithelium can sense signals coming from the central nervous system (CNS) parenchyma via the cerebrospinal fluid (CSF) and through dialogue with circulating immune cells, translate them into a reparative or protective mechanism. Here we found that different T cell populations, with distinct cytokine polarities, accumulate at the CP of healthy animals and affect the immunomodulatory properties of the CP epithelium and its ability to facilitate trafficking of leukocytes. We further show that activation of the CP for expression of trafficking molecules is tightly regulated by two signals; with the first signal coming from the CNS parenchyma, and the second from outside the CNS. Taken together, our findings demonstrate that the CP epithelium is endowed with immunological plasticity allowing it to serve not only as a filter for CSF nutrients, but also as an active interface for selection of circulating immune cells, regulating immune cells trafficking into the CNS.

Regulation of ABC-Transporters by Nuclear Receptors

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Expression and function of ABC-transporters at the blood brain barrier are subject of complex signaling cascades. Nuclear receptors (orphan receptors), such as pregnane X receptor, farnesoid receptor, constitutive androstane receptor, aryl hydrocarbon receptor, glucocorticoid receptor or peroxisome proliferator-actived receptor play a pivotal role in these processes. They respond to internal and external stimuli including metabolites, xenobiotics, pollutants and drugs.To date 48 nuclear receptors have been identified in humans. One of them, PXR, is highly expressed in the BBB. Exposing isolated rat capillaries to the PXR ligands pregnenolone-16-carbonitrile (PCN) and dexamethasone increases p-glycoprotein expression and Pgp–specific transport into capillary lumens. In vivo dosing of rats with PCN and dexamethasone results in increased p-gp expression and function in brain capillaries. In porcine brain capillaries similar results are obtained with PXR substrates rifampicin and hyperforin. A second nuclear receptor at the BBB is AhR, Aryl hydro-arbon receptor, which responds to a large variety of environmental toxins, including DDT or TCDD. In rat capillaries, long term-incubation with AhR-substrates ß-naphtoflavone (BNF) and TCDD results in increased expression of Pgp, Mrp2 and Bcrp, which can be suppressed by AhR antagonist alpha-naphtoflavone. BNF and TCDD effects are also abolished when transcription is inhibited by actinomycin C or translation is inhibited by cycloheximide. Neither actinomycin D nor cyclohexamide by themselves affect transport by ABC-transporters. The results show that ABC transporters at the BBB are target genes of nuclear receptors, which act as cellular xenosensors. Ligand activation of the receptors results in an upregulation of transporters and thus, for substrates that are toxicants or potentially toxic metabolic wastes, increased transporter expression should be protective for the brain.

Pathophysiological impact of hemichannels on the blood-brain barrier transport

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The blood-brain barrier (BBB) plays an essential role in highly selective transport between the circulating blood and the brain. It has been thought that the dysregulation of the BBB transport under the pathological conditions such as stroke, exaggerates the brain damage. Here, we hypothesized that pannexin and/or connexin hemichannels would be involved in the early molecular events of the BBB alterations. Indeed, Thompson *et al*. have shown that hemichannel opening contributes to the profound ionic dysregulation during stroke and may be a ubiquitous component of ischemic neuronal death (*Science* 312:924-927, 2006). Thus, hemichannels present at the BBB might be able to emerge as a promising target for minimizing the neural damage. The purpose of this study is to investigate the protein expression profiles of hemichannels and the hemichannel opening in human brain capillary endothelial cells. We have developed so far LC-MS/MS-based targeted proteomics for human and mouse pannexin and connexin hemichannels by using a series of stable isotope labeled peptides as internal standards. In LC-MS/MS analysis of the human brain capillary endothelial cell line (hCMEC/D3), endogenous peptides for several subtypes of hemichannels were detected. Furthermore, the uptake of model substrates for hemichannels, *i.e*., sulforhodamine 101 and propidium Iodide, by hCMEC/D3 cells was significantly increased in absence of extracellular calcium. The uptake was inhibited by carbenoxolone, a potent hemichannel inhibitor, and a mimetic blocking peptide. These results suggested that the specific types of hemichannels are expressed in human brain capillary endothelial cells and the opening of pannexin and/or connexin hemichannels could alter the BBB transport under pathological conditions, presumably leading to exaggeration of the neural damage.

Blood-brain barrier endothelial amino acid transporters in the control of brain interstitial amino acid homeostasis

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The Blood-Brain barrier (BBB) endothelium expresses 25 or more amino acid (AA) transporters, which to differing degrees regulate transendothelial transport and control brain interstitial fluid (BIF) concentrations. For most AAs, a steep gradient from blood to CSF exists, while concentrations in ECF are assumed to be similar to CSF. However, Glutamine (Gln), the most abundant plasma AA, is almost as concentrated in CSF as plasma. Given that in the CNS, Gln is coupled with the replenishment of the major excitatory and inhibitory neurotransmitters, glutamate and GABA, its levels are also likely regulated in BIF. Our goal is to investigate the AA transporter dependent mechanisms by which the BBB, participates in the regulation of AA BIF homeostasis. Further, since AA transport is known to respond to therapeutic growth factors, such as IGF1, to investigate the response of brain endothelial AA transporter expression and function to IGF1 treatment. For Gln, we hypothesize the Na^+/H^+-dependent AA transporters, Snat3/Slc38a3 and/or Snat5/Slc38a5, play central roles in transendothelial transport since Snat 3 and Snat 5 shuttle Gln both along and against its concentration gradient. In addition, Snat3 is appropriately localized on endothelial membranes to regulate transport. Interestingly we have found that monolayer confluence regulates expression of a number of transporters by hCMEC/D3 (D3) (human *in vitro* BBB cell model) including upregulating Snat3 mRNA. To investigate the *in vivo* contribution of Snat3, we are probing responses by wild type vs. an endothelial targeted Snat3 knockout mouse model using intracerebral microdialysis. In a pilot study, wild-type hippocampal BIF concentrations for several AAs (Ala, Gln, Gly, Ser, Thr) were quantified using the "extrapolation to zero flow rate" method and surprisingly found to be approximately 20% of reported CSF levels. In addition, since the related transporter Snat5 is also co-expressed in BBB endothelial, the kinetics of Snat3 vs. Snat5 transport when expressed seperately or together are being compared using the *Xenopus* oocyte and D3 expression systems. Preliminary data indicates co-expression in oocytes results a non-additive net accumulation of Gln and other AAs suggesting possible regulatory Snat3-Snat5 protein interactions. Furthermore, IGF1, which increases AA transport by microvessels, alters the transcription of a number of AA transporter genes in D3. IGF1 has been reported to have neuroprotective effects following stroke and other neurotramic events. Our data indicates D3 cells may be a reliable *in vitro* model for unraveling the signalling pathways involved in IGF1-mediated effects on the brain microvasculature AA transport.

Astrocytes dysregulate BBB integrity and ABC transporter properties in ALS, a neurodegenerative disease of the motor system

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Amyotrophic Lateral Sclerosis (ALS) is a slowly progressing neurodegenerative disease that selectively affects both upper and lower motor neurons of the nervous system with both genetic (10%) and sporadic (90%) cases. Recent research has implicated ALS as a non-cell-autonomous disease with astrocytes contributing to the toxicity and selective loss of motor neurons. The blood-brain barrier (BBB) is altered in ALS at early disease states and continues to degenerate throughout the course of the disease. This barrier is formed by endothelial cells with closely associated pericytes and astrocytes, which make up the neurovascular unit. Astrocytic end-feet are known to encapsulate more than 90% of the endothelial cells and are instrumental in maintaining the homeostasis of the barrier. Furthermore, ABC drug efflux transporters provide an obstacle to a wide range of neurotoxicants and xenobiotics, as well as limiting the entry of therapeutics into the brain parenchyma. These transporters are predominantly localized to endothelial cells, but they have also been identified on neurons and glial cell surfaces. Recently, our lab identified two transporters, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), with increased expression and function in ALS (Jablonski et al. 2012; Neurobiol. Dis. 47(2):194-200). To further understand the regulation of the tight junction permeability and drug efflux transporter regulation in ALS, we have established a co-culture system whereby primary cultured endothelial cells or bEnd.3 cells were plated on a transwell above a layer of astrocytes. Mutant SOD1-G93A astrocytes were assessed for their contribution to alterations in the endothelial cell barrier. Physiological stressors related to ALS, including H2O2 treatment, contribute to increases in P-gp protein expression and decreases in tight junction protein expression. Mutant SOD1-G93A astrocytes contribute to altered P-gp expression in the endothelial cell layer. This project is an important step in understanding drug efflux transporter regulation in ALS.

A new pathogenic pathway and causative treatment option for Alzheimer's disease – from aging to mitochondrial and ABC transporter dysfunction

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In Alzheimer's disease (AD), the intracerebral accumulation of amyloid-β (Aβ) peptides is a critical yet poorly understood process. Aβ clearance via the blood-brain barrier is reduced by ~30% in AD patients, but the underlying mechanisms remain elusive. ATP-binding cassette (ABC) transporters have been implicated in the regulation of Aβ levels in the brain. Using new genetically modified mouse models, we show for the first time that the transporter ABCC1 has an exceptional functional impact on cerebral Aβ clearance and accumulation. In mouse models, a deficiency of ABCC1 increases cerebral Aβ levels up to 12-fold, whereas activation of ABCC1 using an FDA-approved drug significantly reduces Aβ load. Furthermore, we highlight functional abrogation of mitochondrial oxidative phosphorylation (OXPHOS) during aging as the important physiologic mechanism leading to ABC transporter dysfunction in elderly.
Thus, by altering the temporal aggregation profile of Aβ, □□pharmacological activation of ABC transporters and OXPHOS could impede the neurodegenerative cascade during aging that culminates in the dementia of Alzheimer's disease.

Optimisation and functional characterisation of a 'new' porcine brain endothelial cell model of the BBB

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There has been a long history of development of *in vitro* blood-brain barrier (BBB) models, since the earliest successful isolation and growth of brain endothelial cells in culture. The aim has always been to generate models that mimic as closely as possible the *in vivo* BBB phenotype, however, this has proved difficult. The most successful and complete models are those based on primary cultured brain endothelial cells, that give a good yield of cells per brain, show minimal contamination by other cell types, preserve good tight junction structure and show a high transendothelial electrical resistance (TEER); this is associated with pronounced apical-basal polarity and expression of relevant (polarized) BBB transporters and receptors. In practice only bovine and porcine brain preparations have so far provided cells of this quality and quantity.
We have adopted a porcine brain endothelial cell (PBEC) model initially developed by Louise Morgan at Eisai London ~1994, and modified the method to include improvements based on current understanding, including puromycin to kill contaminating pericytes and differentiating factors to enhance the BBB phenotype. We will present details of this method, variants that replicate some aspects of the *in vivo* neurovascular unit, and characterization of the models generated, with particular attention to features that give evidence for preservation of a functional BBB phenotype.

Assessing the free brain/free plasma ratio in vitro in early drug discovery

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The value of many promising CNS drug candidates is diminished by the presence of the Blood-Brain Barrier (BBB) that prevents many neuropharmaceuticals from eliciting a desired pharmacological effect at an attainable dose. In the early 90's, we have established an *in vitro* model of the BBB by co-culturing bovine brain capillary endothelial cells together with rat glial cells. This model has been successfully used for screening and mechanistic purposes in a number of major pharmaceutical companies for more than a decade. Historically, the focus has been to use these kinds of *vitro* models to optimize rate of drug delivery to the CNS, whereas *in vivo* brain/plasma ratios were used for optimizing extent.
Since it is thought that only the free brain concentration (Cu,br) is available for interaction with the majority of CNS receptors it may be essential to also determine this parameter for CNS compounds. Therefore, we have modified the use of our current in vitro model of the BBB so that, in addition to permeability, it could also generate a quantitative parameter such as the free brain/free plasma ratio. These information could help prioritizing test compounds in the pharmacological field. As from a knowledge of free plasma concentration (Cu,pl) and *in vitro* efficacy (IC50 or EC50), the estimation of the steady state Cu,br/Cu,pl generated by the *in vitro* model can be used to make early predictions about the likelihood of achieving a pharmacological effect in the CNS.

Comparison of epithelial cell line-based surrogate and brain endothelial cell-based blood-brain barrier models for drug screening

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Predicting blood-brain barrier (BBB) penetration and blood-brain partitioning of new chemical entities is a major need in pharmaceutical research. Currently, an industry-standard BBB drug penetration model is not available. We compared the morphology, functionality and gene-expression profile of a brain endothelial BBB model (EPA: triple culture of brain endothelial cells with pericytes and astrocytes) and the epithelial cell-based (native Caco-2, high P-glycoprotein expressing vinblastine-treated VB-Caco-2 and MDCK-MDR1) surrogate BBB models.The cytoarchitecture of the endothelial and epithelial models differed in several major points which may have a relevance in drug tansport. The gene expression profile of tight junction proteins, efflux and SLC transporters and metabolic enzymes were also distinct in the model types. The expression level of occludin was high in all models, but each model type expressed a unique claudin pattern. Major BBB efflux (P-glycoprotein) and influx transporters (GLUT-1, LAT-1) were present in all models. However, the absence of BCRP, MRP-6, -9, MCT-2, -6, PHT-2, OATP-1 and -2, GAT-1 GABA and NET norepinephrine transporters in one or both types of epithelial models suggests that the brain penetration of their substrates can not be predicted using Caco-2 or MDCK models. The strong expression of efflux transporter genes not present at the BBB, like MRP-2 can contribute to the incorrect estimation of the brain penetration of drugs, like vinblastine. All models presented tight paracellular barrier to measure drug permeability. Using a set of 10 compounds the EPA model gave the highest correlation with in vivo data. Epithelial models also gave good estimates for passive compound penetration and a better prediction for efflux pump substrates than the EPA model. High influx was measured for 10 SLC ligands on the EPA model, except for substrates that have considerable parallel efflux transport, like gabapentin, salicylic acid, and atorvastatin. In conclusion: VB-Caco-2 and MDCK models give good predictions for passive diffusion and efflux pump ligands, but have epithelial morphology and gene-expression, and do not express several BBB efflux and SLC transporters. The cytoarchitecture, functionality, complexity and gene expression profile of the EPA model is the closest to the in vivo BBB. The sequential and selective use of the epithelial and BBB models is suggested as the best screening protocol for the pharmaceutical industry.

Cellular Response to *Neisseria meningitidis* in a Human Model of the Blood-Cerebrospinal Fluid Barrier

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The human-specific, Gram-negative bacterium *Neisseria meningitides* (*Nm*) is a leading cause of bacterial meningitis world-wide. It has been described that *Nm* can enter the central nervous system via the blood-cerebrospinal fluid barrier (BCSFB), which is constituted by the epithelial cells of the choroid plexus. Using a recently established *in vitro* model of the BCSFB based on human choroid plexus papilloma epithelial cells (HIBCPP) we have investigated the cellular response of HIBCPP challenged with the pathogenic *Nm* strain MC58. In comparison we analysed the response to the closely related unencapsulated carrier isolate *Nm* alpha14. Transcriptome analysis revealed a considerable stronger transcriptional response after infection with the pathogenic strain, particular with the capsule-deficient mutant MC58siaDmut, which correlated with bacterial invasion levels. Expression evaluation and Gene Set Enrichment Analysis pointed to an NFkappaB-mediated pro-inflammatory immune response involving upregulation of the transcription factor IkappaBtheta. Measurement of cytokine production by infected HIBCPP employing cytometric bead arrays and ELISA detected and confirmed, among others, the production of IL8, CXCL1-3 and the IkappaBtheta target gene IL6. The expression profile of pattern recognition receptors in HIBCPP and the response to specific agonists indicates that TLR2 rather than TLR4 is involved in the cellular response following infection with *Nm*.

Infection with echovirus 30 - effects on leukocyte migration across the blood-cerebrospinal-fluid barrier in a human in vitro model

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Enterovirus is the most common pathogen causing viral meningitis especially in children. The choroid plexus, which forms the blood-cerebrospinal-fluid (CSF) barrier (BCSFB), was shown to be involved in the pathogenesis of enteroviral meningitis. In a human *in vitro* model of the BCSFB consisting of human choroid plexus papilloma cells (HIBCPP), the permissiveness of plexus epithelial cells for Echovirus 30 (EV30) in the multiplicity of infection (MOI) 10 and its effects on transepithelial immune cell migration was analyzed. HIBCPP could be directly infected by EV30 from the apical as well as from the physiological relevant basolateral side (results generated by immunoblotting and quantitative real time PCR (q-PCR). During the infection no alterations of barrier function could be observed. Analysis of transepithelial transmigration of T lymphocytes and polymorph nuclear granulocytes (PMN) across the *in vitro* model of the BCSFB in the absence or presence of CXCL12 (for T lymphocytes) or IL8 (for PMN) after infection with EV30 revealed a significant transepithelial migration of naïve T lymphocytes and PMN in response to CXCL12 and IL8 stimulation, respectively. EV30 infection alone had only a minor effect on the transmigration rates but lead to a reduced cell viability of PMN but not of T lymphocytes, which might explain the low transmigration rate of PMN. Addition of CXCL3 in the presence and absence of IL8 lead to a significant increase of transepithelial PMN migration. Consequently, HIBCPP constitutes a valuable human model to study viral infection at the BCSFB. PMN transmigration across the BCSFB is enhanced by CXCL3.

Molecular Regulation of Endothelial Blood-Brain Barrier Function in Health and Disease

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Tight control of vascular permeability is essential for normal brain function but little is known about the molecular basis of blood-brain barrier (BBB) development and maintenance.
We have shown that endothelial Wnt/β-catenin signaling is necessary for the induction of BBB characteristics. β-Catenin signaling in primary brain endothelial cells in vitro induced claudin-3 expression and BBB-type tight junction formation, as well as a BBB characteristic gene signature, including upregulation of glucose transporter 1 (Glut1). However, so far it is not known in detail if and how this pathway affects other BBB-related genes.
Interestingly, CYP1B1, a member of the cytochrome P450 (CYP) proteins, is strongly expressed in brain endothelial cells (ECs) and is supposed to be involved in the detoxification of xenobiotics at the BBB. Its transcriptional regulation in ECs however, has not been evaluated in detail so far.
Affymetrix® transcriptome analysis revealed a significant downregulation of CYP1B1 in endothelioma cells lacking β-catenin compared to wild-type (WT) controls, which could be confirmed by quantitative RT-PCR. Stimulation of primary brain ECs with Wnt3a, leading to the transcriptional activation of β-catenin, resulted in CYP1B1 upregulation. Furthermore, in subconfluent ECs, as well as in ECs lacking VE-cadherin, in which β-catenin signaling has been reported to be increased, CYP1B1 expression is also augmented. In conclusion, these data suggest that β-catenin participates in the regulation of CYP1B1 in ECs in general and in BBB ECs in particular. Therefore, the Wnt/β-catenin pathway likely contributes to the regulation of a broad range of BBB-specific genes including CYP1B1.

Detection of tight junction strand morphologies of claudins and tight junction associated marvel-proteins (TAMPs)

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Sealing of the paracellular cleft by tight junctions is of central importance for endothelia, such as in the blood-brain barrier, to function as efficient barrier between the circulation and the CNS. Claudins represent the major tight junction (TJ) component involved in establishing this barrier function. The TAMPs, such as occludin (Occl), tricellulin (Tric) and marvelD3 (MD3), belong to a family of tetraspan transmembrane proteins carrying a marvel domain. Neither the intrinsic function of the TAMPs nor its interactions with the claudin family is fully understood. To investigate the tight junction strand network of claudins alone or cotransfected with occludin family proteins, we used the TJ-free cell-system HEK-293. We demonstrated strong interactions of claudin-1 (Cld1) with the TAMPs by fluorescence resonance energy transfer. Furthermore, the coexpression of Occl or Tric with Cld1 led to an immobilization of Occl or Tric at the plasma membrane, shown by fluorescence recovery after photobleaching experiments. To get a precise insight into the molecular principle of the interactions we performed freeze-fracture electron microscopy. The coexpression of Tric, Occl or MD3 with Cld1 exhibited a more compact tight junction mesh network, which is comparable to the strand network of functional tight junctions (e.g., in the BBB). These studies demonstrate a high dynamic behavior of the heterophilic interactions within the tight junction protein families.

The Structure and Function of Septate Junctions

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In general, occluding junctions between epithelial cells are the tight junctions (TJs) in vertebrates and the septate junctions (SJs) in invertebrates. SJs are present in endodermal and ectodermal epithelia, including various 'blood barriers' (brain, eye, testes). Whereas tight junctions present membrane fusions of adjacent cells, septate junctions (SJs) resist membrane fusions with ladder-like septa that maintain a paracellular cleft of about 20 nm. The cleft offers a route for transepithelial permeation. Consistent with a high water permeability of the cleft is the secretion of a fluid by Malpighian (renal) tubules of insects that is isosmotic with the peritubular medium under both control and diuretic rates. In contrast, SJs are permselective and variably permeable to solutes. When the tubules secrete fluid in vitro under control conditions, the barrier is largely impermeable to the three major osmolytes of secreted fluid: Na, K and Cl, reflecting the barrier function of SJs. However, when the tubules are treated with the diuretic hormone aedeskinin, SJs become highly permeable to Cl. The switch from a Cl-impermeable to a Cl-permeable SJ is immediate and reversible upon washout of aedeskinin, indicating a post-translational mechanism for regulating the paracellular Cl permeability. The molecular components of SJs include many proteins that are homologous with proteins associated with TJs in vertebrates, but the proteins that form the septa in SJs are unknown. Hypothetically, these proteins maintain a paracellular geometry suitable for high rates of transepithelial water flow. In addition, TJ-like proteins provide the barrier- and channel-like properties for solutes.

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PCB153 affects tight junction proteins in ovine choroid plexus

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Ortho-substituted polychlorinated biphenyls (PCBs) constitute a large part of PCBs residues found in the environment and animal tissues; most of the congeners accumulating in brain are ortho-substituted. We demonstrated that the same oral dose of PCB153 induced higher concentrations in the cerebrospinal fluid (CSF) of treated ewes than in controls during short days (SD), while no differences were noted during long days (LD). The mechanisms involved remain unknown, but changes in the permeability of blood-CSF barrier located in the choroid plexus (CP) constitute a tenable hypothesis. The present study analyzed the effect of PCB153 on the expression of tight junction (TJ) proteins: occludin, claudin-1, -5, junctional adhesion molecule-1 (JAM-1), zona occludens (ZO)-1, ZO-2, ZO-3, afadin (AF-6) in ovine CP during LD and SD. The CP was collected from ewes in which we observed photoperiodic effect on PCB153 concentration in the CSF. These ewes (ovariectomized and estradiol treated) were treated with PCB153 (per os, 0.33 mg/kg/day, 3 times a week for 3 consecutive weeks) or with vehicle (control) during LD and SD.
Exposure to PCB153 affected TJ proteins only during SD, when levels of claudin-1, ZO-2, and AF-6 were significantly lower compared to control ewes. No differences were observed for occludin, JAM-1, claudin-5, ZO-1 and ZO-3. There was no effect of PCB153 treatment on the TJ mRNA levels. Results indicate that PCB153 mediates selective alteration of TJ proteins in blood-CSF barrier. These alterations seem associated with the level of PCB153 in blood, which is photoperiodically-modulated. This highlights the importance of photoperiod in the susceptibility of sheep to PCB and emphasizes the importance to consider the photoperiod parameter when analyzing in vivo effect of PCBs in regard with metabolism especially in species where this latter is modulated by photoperiod.

Elucidating the molecular organization of tight junctions strands

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Paracellular barrier properties of tissues are mainly determined by the composition of claudin heteropolymers. For the BBB, Claudin-5 (Cld5) is essential for the tightening against small molecules and copolymerization of Cld5 and Cld3 is thought to determine the extremely tight barrier properties of the BBB. Modulation of the BBB is desirable, on the one hand to stabilize the paracellular barrier under pathological conditions and on the other hand to transiently open the BBB to improve drug delivery. In order to develop strategies for modulation of the BBB, we analyzed the molecular mechanism of the interaction between claudins and of the barrier formation. We generated Cld3/Cld5 chimeras and reconstituted TJ-strands by transfection of HEK293-cells and L-fibroblasts with Cld3, Cld5 and/or chimeras. We analyzed their homo-/heterophilic cis- and trans-interactions by quantification of colocalization, cell-contact enrichment scanning, fluorescence resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP). Furthermore, we investigated their oligomerization state with Blue Native electrophoresis. The ability of the chimeras to form TJ-strands and the strand morphology was studied by freeze fracture electron microscopy and a novel super resolution localization microscopy approach. The barrier function of the TJ-strands was demonstrated by fluorescent tracer imaging assays. Residues critically involved in the interactions of the claudins were identified by site directed mutagenesis In summary, our data provide novel molecular insights into the assembly of tight junctions and their accessibility for pharmacological intervention.

Functional aging of the brain reflects epithelium-T cell crosstalk at the blood-CSF barrier

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Circulating immune cells have been repeatedly shown to be essential for central nervous system (CNS) maintenance. Specifically, T cells that recognise CNS antigens were shown to contribute to the functional integrity of the CNS under both normal and pathological conditions, mediating hippocampus-dependent learning and memory, adult neurogenesis, and neurotrophic factor production. Nevertheless, T cells can rarely be found in the healthy CNS parenchyma – raising several key questions as to how, where and when these cells exert their effects on the healthy CNS. In this study, we developed a novel tool involving deep sequencing analysis and tailor-made bioinformatics to identify the specificity of the T cell receptor (TCR) repertoire in specific tissues. We show that the choroid plexus epithelium of the blood-CSF barrier is an active interface between the blood and the brain that is constantly populated by CNS-specific effector memory CD4+ T cells. In aged mice we found the immune-epithelial crosstalk in this compartment to be dysregulated, critically affecting the choroid plexus epithelium. Partial restoration of cognitive ability in old mice, by homeostatic-driven proliferation of memory T cells, resulted in immunomodulation of the CP and hippocampal plasticity. Thus, aging of the brain might reflect a malfunction of its interface with the immune system, a target amenable to immunomodulation for arresting cognitive decline.

In vivo and *in vitro* effects of Nrf-2 inducing substances on blood-brain barrier tight junction proteins

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The transcription factor, nuclear factor E2-related factor 2 (Nrf-2) has been suggested to be important for the integrity of the blood-brain barrier (BBB), a mechanism that is supposed to be mediated via the upregulation of cytoprotective genes such as NADPH quione oxidoreductase (NQO1). The aim of this study was to investigate published or suggested Nrf-2 inducers on brain endothelial tight junction (TJ) proteins, *in vitro* as well as *in vivo*.
Two endothelial cell lines were used: the Madin Darby canine kidney II cell line (MDCKII) and the human brain endothelial capillary cell line (hCMEC/D3). These cells were treated with four compounds including monomethylfumarate (MMF), dimethylfumarate (DMF), sulphoraphane (SFN) or tert-buthylhydroquinone (tBHQ), with pre- or post-stimulation of cells with tumor necrosis factor alpha (TNF alpha), known to disrupt the BBB. Changes in the expression level of Nrf-2, NQO1 and TJ proteins (claudin-5 (Cl-5), occludin and zonula-occludens-1) were evaluated by Western blot and real-time PCR. In the hCMEC/D3 cells at resting state, there was only an upregulation of Nrf-2 when cells were incubated with DMF or tBHQ. TNFalpha treatment alone led to a significant decrease in Nrf-2 and NQO1 on both mRNA and protein levels. This down-regulatory effect of TNFalpha was reversed by pre- treatment with MMF or tBHQ, only Nrf-2 is upregulated on both levels.
To study the impact of DMF on BBB endothelium *in vivo*, DMF (15 mg/kg/day) was given orally to C57BL/6 mice suffering from myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis (EAE). On day 72 of EAE the number of claudin-5 positive TJ was significantly reduced in EAE mice as compared to healthy mice. Yet, this number was not significantly different between DMF and saline treated EAE mice.
In summary, Nrf-2 inducing compounds neither displayed *in vitro* nor *in vivo* effects on BBB TJ proteins.

The impact of extracellular matrices on the barrier function of cerebral endothelial cells

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The blood-brain barrier (BBB) is composed of the cerebral microvascular endothelium which is mainly build by astrocytes, pericytes and the extracellular matrix (ECM). It is commonly accepted that all these different cell types contribute to the BBB-extracellular-matrix (ECM) and secrete a different pattern of matrix proteins (e.g. laminin and fibronectin) as well as their own storage of growth factors. This inductive environment enables the ECM to modulate various aspects of cellular differentiation including cell proliferation and to mediate the highly specific barrier phenotype. To elucidate the role of astrocyte- and pericyte-derived endogenous extracellular matrices in improving the integrity of the capillaries we developed a new generation model in vitro. For this generation model several generations of cells of the BBB were used for ECM production. By cultivating porcine brain capillary endothelial cells (PBCEC) on different endogenous matrices of cells of the BBB, it was demonstrated that the improvement of BBB-properties correlates with the endogenously isolated ECM and their special composition of matrix proteins. By using different endogenous cell specific ECMs (e.g. pericyte-derived ECM) an upregulation of all tight junction (-associated) proteins e.g. occludin, claudin-5 and ZO-1 on mRNA and protein level has been shown on PBCEC. Specific structural differences and composition of matrix proteins of the ECM of cells of the BBB has been analyzed by transmission electron microscopy (TEM) and enzyme-linked immunosorbent assay (ELISA). To understand the influence of inductive matrix proteins of cells of the BBB will help to better understand factors influencing BBB integrity, function and maintenance.

The properties of brain endothelial cells in an in vitro model of Moyamoya disease

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The Moyamoya disease (MMD) is a rare cerebrovascular disorder of unknown etiology among the Caucasian population, characterized by spontaneous occlusions of vessels of the circle of Willis and its main branches. MMD patients develop an aberrant network of collateral circulation vessels leading to hemorrhagic and ischemic strokes. The MMD progression has been attributed to some growth and angiogenic factors promoting migration and proliferation of circulating endothelial progenitors, smooth muscle and endothelial cells (ECs). However the molecular background of fragile vessel development in MMD still needs to be elucidated.
Here we propose an in vitro model of the blood-brain barrier, a murine brain EC line, to examine the properties of ECs treated with MMD sera. In particular we compared the endothelial sealing of cultures incubated with serum from healthy controls, MMD and arteriosclerosis patients. First we analysed the transendothelial resistance in brain ECs treated with MMD serum. This resulted in a strong increase of the monolayer permeability compared to healthy controls. Subsequently, we examined the expression of tight and adherens junctions of the brain endothelium and detected Claudin-3, Claudin-5, Occludin and VE-cadherin being significantly reduced after incubation with MMD sera, compared to healthy and arteriosclerotic controls. Furthermore we measured an induction of intra- and extracellular levels of Angiopoietin2, a barrier destabilizing angiogenic factor, where the expression of its antagonist, Angiopoietin1, was decreased. Beside these results we also confirmed an upregulation of matrix metalloproteinase-9 and VEGF-A in ECs incubated MMD sera. Summarizing, the pathophysiological processes on a molecular level in MMD still need to be clarified in detail. In this regard, we introduce a brain EC line as an in vitro tool providing a new insight into molecular processes affecting the EC barrier sealing in fragile MMD vessels. We propose the enhancement of endothelial plasticity, induced by an imbalance of angiogenic factors, proteins of the junctional complex and of the extracellular matrix, may play a key role in the formation of occlusive lesions and edema in MMD.

Lipopolysaccharide-induced fever depends on prostaglandin E2 production specifically in brain endothelial cells

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Immune-induced prostaglandin E2 (PGE2) synthesis is critical for fever and other centrally elicited disease symptoms. The production of PGE2 depends on cyclooxygenase-2 and microsomal prostaglandin E synthase-1 (mPGES-1), but the identity of the cells involved has been a matter of controversy. We generated mice expressing mPGES-1 either in cells of hematopoietic or non-hematopoietic origin. Mice lacking mPGES-1 in hematopoietic cells displayed an intact febrile response to lipopolysaccharide, associated with elevated levels of PGE2 in the cerebrospinal fluid. In contrast, mice that expressed mPGES-1 only in hematopoietic cells, while displaying elevated PGE2 levels in plasma but not in the cerebrospinal fluid, showed no febrile response to lipopolysaccharide, thus pointing to the critical role of brain-derived PGE2 for fever. Immunohistochemical stainings showed that induced cyclooxygenase-2 expression in the brain exclusively occurred in endothelial cells, and qPCR analysis on brain cells isolated by flow cytometry demonstrated that mPGES-1 is induced in endothelial cells, and not in vascular wall macrophages. Similar analysis on liver cells showed induced expression in macrophages and not in endothelial cells, pointing at the distinct role for brain endothelial cells in PGE2 synthesis. These results identify the brain endothelial cells as the PGE2-producing cells critical for immune-induced fever.

Alternative Approach for Treatment of Metachromatic Leukodystrophy Disease with Enzyme Replacement Therapy by Crossing the Blood-Brain Barrier

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The lysosomal storage disease metachromatic leukodystrophy (MLD) is a terminal illness, caused by arylsulfatase A (ASA) deficiency. An accumulation of its substrate acidic 3-O-sulfogalactosylceramide leads to demyelination and degradation of neurons and glial cells. Patients have progressive motoric and mental disorders. Moreover, the lifespan dramatically is reduced. Enzyme replacement therapy (ERT) is a promising approach for MLD, but is limited by the blood-brain barrier (BBB). In the BBB the claudins 1, 3, 5 and 12 are expressed. Different members of the claudin protein family are known to be modulated by certain peptides and proteins, e.g. by the C-terminal region of CPE (*Clostridium perfringens* enterotoxin; cCPE). It was shown to bind claudin-3 and -4, thereby increasing the paracellular permeability of tissue barriers. cCPE or other Claudin binding peptids could be used to overcome the BBB and to deliver recombinant human ASA to the brain tissue.
Our aim is to investigate cCPE-binding and -endocytosis as well as the effect of claudin modulation on the permeability for macromolecules of brain endothelial cell monolayers. Therefore, recombinant cCPE and peptids are used for the investigation of endocytosis in Cld3-expressing cell lines by confocal microscopy and for permeability assays. Furthermore, the expression and purification of an ASA-cCPE fusion protein as basis for ERT was established in stably transfected HEK-293 cells. Currently, we are analysing the ability of ASA-cCPE to bind and cross endothelial cells by the transcellular pathway.

Modulation of paracellular barrier properties using claudin-mimetic proteins and peptides

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The paracellular cleft within epithelia and endothelia is sealed by tight junctions (TJ) which control the flow-through of molecules, *e.g.*, pharmaceuticals through tissue barrier. For this reason, the modulation of the TJ integrity is a promising strategy to improve the drug delivery. Claudins (Cld) form the backbone of the TJ. They consist of four transmembrane helices as well as two extracellular loops (ECL) which are involved in the paracellular barrier function. Claudin-claudin interactions are thought to be based on the ECL, which also can bind peptide ligands such as *Clostridium perfringens* enterotoxin (CPE). As the receptor of CPE, the ECL2 in Cld3 and -4 was identified [1]. After application of the C-terminal region of CPE (C-CPE) on MDCK-I (Madin Darby canine kidney) cells, a reduction of the TJ-barrier was observed [2]. Nothing is known whether peptide sequences out of the ECL may interact with the ECL of the full length claudins. Our approach is to use recombinant claudin proteins and synthetic peptides, which interact with claudins, to reduce the barrier integrity. These interactions could be a hint for a selective and transient modulation of the TJ barrier. We demonstrated that different recombinant proteins, designed from the first ECL of Cld1 and -5, formed monomers and dimers. The dimers were prevented by reducing agents, indicating a redox sensitivity of this binding. The characterization of the secondary structure of the ECL1 of Cld1 showed a mixed structure with mainly α-helical and, to a lower extent, β-sheet segments when analysed in phosphate buffer. The addition of SDS, a β-sheet supporting agent, doubled the β-sheet content, whereas TFE, which supports helix formation, showed no effect on the helicity. Binding studies by means of pulldown assays and microscale thermophoresis showed a high affinity binding of the recombinant ECL1 constructs of Cld1 to full-length Cld1 (Kd: 7.1 nM $\pm$ 0.36 nM) and also to a Cld1-derived peptide mC1C2 (Kd: 9.3 nM $\pm$ 0.75M). Summarizing, we state that the ECL1 is a flexible structure, which can bind to itself and to full length claudins, which might modulate the integrity of the TJ.

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Oxidative Stress in the Blood Brain Barrier Induced by Numerous Toxins and Its Consequences

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Free radical production and the resulting oxidative stress play an important role in the pathogenesis of numerous neurodegenerative disorders. These include Parkinson`s disease, Alzheimer`s disease, and various forms of dementia. Recent data indicate that incidences of neurodegenerative disorders are on the rise. This is mainly due to the increased toxins in our environment and increased longevity. Under physiological conditions, the brain is well protected by the blood brain barrier (BBB). The BBB is the capillary bed of the brain that is modified by having the adjacent cerebrovascular endothelial cells joined by intercellular tight junctions. These junctions and other endothelial cell modifications result in a barrier between blood and the brain interstitial fluid that tightly regulates the exchange of substances between the brain and blood. The BBB helps to maintain the homeostatic environment of the brain and supplies the brain's nutritive needs. Under physiological conditions, the integrity of the BBB is protected from oxidative stress because of its high levels of antioxidant enzymes. These peroxide-detoxifying enzymes help protect the functional integrity of the BBB, which is essential for the brain to function properly. If “toxins” increase free radical production, then antioxidant protection of the BBB may fail and oxidative stress manifests in the BBB. Consequently, the BBB’s pivotal function, “protecting the brain”, will be lost, resulting in neurodegenerative disorders. Environmental toxins, toxic viral proteins and addictive drugs may induce oxidative stress and disrupt the integrity of the BBB. This presentation will provide an overview of the oxidative effects of nanoparticles, diesel exhaust particles, toxic HIV-1 proteins, and methamphetamine in the BBB. The highlights of our recent studies of a potent antioxidant to protect the BBB against oxidative stress will also be presented.

CNS targeted anti-inflammatory agent reduces pathology in mouse model of ALS

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The progressive and fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS) can be modelled in transgenic mice overexpressing mutant superoxide dismutase-1 (*SOD1*^{G93A}). The disease profile involves onset of motor symptoms at ~90 days (d), progressing to death at 130-140 days. The disease is characterized by loss of spinal cord motor neurons and associated neuroinflammation. The blood-brain barrier permeability is unchanged, even later in the disease, therefore access for larger therapeutic compounds is limited. Specifically targeting the central inflammatory component of this process, through the use of a CNS-targeted liposomally-packaged therapy, might reduce motor neuron loss and delay or limit the behavioural component of the disease.

Methods: Mice were divided into 4 treatment groups (i) Wild type (WT) + saline; (ii) SOD1 + saline, (iii) SOD1 + free methylprednisolone (MP), (iv) SOD1 + glutathione PEGylated liposomal MP (2B3-201). Animals were treated once a week with intravenous injections from 60-116d of age. Prior to each treatment animals were weighed and motor function was tested with a rotor-rod, according to standard procedures. Mice were imaged at 120d using T2-weighted MRI to detect signal intensity in brainstem nuclei (V, VII and XII). Subsequently, mice were terminally anaesthetised and perfusion-fixed for histology. Brain tissue was cryo-protected in 30% sucrose, frozen in OCT and cut at 10-m. Sections were stained using H&E, and standard immunohistochemistry for astrocytes (GFAP) and microglia (Iba1).

Results: All animals showed a progressive increase in body weight during the experimental period, with no significant differences between treatments. SOD1 animals showed a significantly lower rotor-rod score compared to WT animals, as early as 60d. All SOD1 groups showed a significant decrease in motor performance from ~100d, however 2B3-201-treated animals showed a significantly slower decline compared to free MP treated animals. SOD1 animals showed a significant increase in signal intensity on T2 weighted MR images, which may reflect the combination of neuronal vacuolation and glial activation in these motor nuclei. Both treatments reduced T2 hyperintensity, but a greater effect was observed with 2B3-201 than free MP.

Conclusions: Both free and packaged steroid reduced brainstem pathology, but the effect was more pronounced for 2B3-201 than free MP. The CNS-targeted anti-inflammatory agent 2B3-201 has therapeutic potential in ALS.

An allograft glioma model reveals the dependence of aquaporin-4 expression on the brain microenvironment

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Aquaporin-4 (AQP4), the main water channel of the brain, is highly expressed in animal glioma and human glioblastoma in situ. In contrast, most cultivated glioma cell lines don't express AQP4, and primary cell cultures of human glioblastoma lose it during the first passages. Accordingly, in C6 cells and RG2 cells, two glioma cell lines of the rat, and in SMA mouse glioma cell lines, we found no AQP4 expression. We confirmed an AQP4 loss in primary human glioblastoma cell cultures after a few passages. RG-2 glioma cells if grafted into the brain developed AQP4 expression. This led us consider the possibility of AQP4 expression depends on brain microenvironment. In previous studies, we observed that the typical morphological conformation of AQP4 as orthogonal arrays of particles (OAP) depended on the extracellular matrix component agrin. In this study, we showed for the first time implanted AQP4 negative glioma cells in animal brain or flank to express AQP4 specifically in the intracerebral gliomas but neither in the extracranial nor in the flank gliomas. AQP4 expression in intracerebral gliomas went along with an OAP loss, compared to normal brain tissue. AQP4 staining in vivo normally is polarized in the astrocytic endfoot membranes at the glia limitans superficialis and perivascularis, but in C6 and RG2 tumors the AQP4 staining is redistributed over the whole glioma cell as in human glioblastoma. In contrast, primary rat or mouse astrocytes in culture did not lose their ability to express AQP4, and they were able to form few OAPs.

Oleic acid increases permeability of blood brain barrier in the rat brain

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BBB maintains the cerebral microenvironment in a tight manner by limiting transport of substances across BBB. Tools which can regulate BBB permeability will be valuable for the drug delivery into the brain. In the previous works, we demonstrated intra-arterially administered fatty acid induced reversible dysfunction of BBB using MRI. We investigated the cellular mechanism of enhanced BBB permeability in this study. Oleic acid induced BBB alteration was visualized by leakage of Evans blue or FITC-labeled dextran and quantitated by measuring Evans blue concentration in the brain tissue and counting EBA immunoreactivity. Induction of ICAM-1 in the vascular endothelial cells, infiltration of neutrophils into the brain parenchyma, induction of iNOS and production of nitrotyrosine and MDA were observed in oleic acid treated brain. Oleic acid induced MMP-2, -3, -9 and -13 in the vessels and MMP-9 in the brain parenchyma. Brain edema and increase of AQP4 was significant in the oleic acid treated brain. iNOS inhibitor suppressed oleic acid induced Evans blue leakage and loss of EBA immunoreactivity. In conclusion, we suggest that nitric oxide production by iNOS is one of the contributing factors of oleic acid induced BBB disruption.

Reduced intrathecal IgG synthesis in the cerebrospinal fluid from MS patients on natalizumab therapy

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Oligoclonal bands (OCB) are qualitative evidence of a central nervous system (CNS)-restricted (intrathecal) IgG production and a hallmark finding in the diagnosis of multiple sclerosis (MS). The monoclonal antibody natalizumab (NZB) is targeted to interfere with the migration of inflammatory immune cells across the blood-brain barrier into the CNS. Recently, OCB have been reported to disappear from the cerebrospinal fluid (CSF) in 4 out of 6 NZB-treated MS patients. We calculated the fraction of intrathecally produced IgG (IgGIF) in our patient cohort and comparatively analyzed the quantitative (IgGIF) and qualitative data (detection of OCB) to investigate a potential NZB effect on CNS-restricted humoral immune activities in MS.

IgGIF in % of total IgG from 32 MS patients were calculated according to Reiber (1998). Detection of OCB was performed by isoelectric focussing. CSF data were available from lumbar punctures (LP) performed at first diagnosis (baseline, n=18) and/or for exclusion of opportunistic infection in case of disease exacerbation during NZB therapy (follow-up, n=13).

Quantitation of CNS-restricted IgG showed a reduced IgGIF ($p=0.0026$) in NZB-treated patients compared to MS patients at first diagnosis. Qualitative evidence of OCB did not differ significantly between groups. Intraindividual comparisons of baseline and follow-up data showed a reduced IgGIF ($p=0.018$, n=6) and disappearance of OCB in 4 out of 13 patients ($p<0.003$).

We show a pronounced reduction in the IgGIF and, to a lesser extent, disappearance of OCB in NZB-treated MS patients. This strongly suggests effectiveness of NZB therapy behind brain barriers. Prospective studies and clarification of the underlying mechanisms should receive further attention since they might provide important knowledge about the clinical relevance of this finding and the occurrence of opportunistic CNS infections during NZB therapy.

The aged rat – a model for Alzheimer's Disease?

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Age is an important risk factor in many neurodegenerative diseases. During ageing, multitude of biological and biochemical processes undergo changes, contributing to disease progression.
One of the most prevalent age-related neurogedenerative diseases is Alzheimer's Disease (AD), currently affecting 25 million people world-wide and prognosed to increase to 100 million cases in 2050.
By far the most commonly used preclinical models for AD are the amyloid precursor protein (APP) - overexpressing transgenic mouse models (TgAPP), which mimick the accumulation of toxic amyloid-ß leading to, amongst others, plaque depositis in the brain. Although these mouse models exert some pathogenic features of AD, e.g. like amyloid-ß related pathology and some cognitive deficits, they are also highly variable and focus only on the amyloid beta cascade hypothesis of AD, omitting other disease aspects like tau pathology, BBB integrity and metabolism changes, and most importantly, aging. Indeed, the TgAPP mice models mimick only familial AD, and not the far more common sporadic, age-related AD. Given that TgAPP mouse models have the above-mentioned limitations, we sought to explore other models. One such model is the aging rodent (rat) model. This model bears the benefit that repeated, larger volume CSF samples can be drawn which aids in the development of translatable and PK/PD biomarkers. A second benefit is the option to examine multiple measurements within the same sample due to higher sample volumes. As such, we examined several markers in CSF of young and aged rats to establish a baseline measurement of albumin, pyruvate and amyloid-ß. Together with hemoglobin content, which served as control for blood contamination. In a separate group of animals we examined both cognitive performance and neuronal plasticity. The results of these experiments as well as it implications will be discussed.

Repetitive hyperthermia-induced seizures in early life alter blood-brain barrier integrity and seizure thresholds in rats with cortical dysplasia

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Febrile seizures in individuals with neocortical malformations have been associated with the development of temporal lobe epilepsy (TLE). Impairment of the blood–brain barrier (BBB) after seizures may have contributed to the occurrence of TLE, however the underlying mechanism(s) is not yet clear. The purpose of this study was to determine whether repetitive hyperthermia-induced seizures in the immature rat with cortical dysplasia (CD) change BBB integrity and seizure susceptibility. Repetitive hyperthermia-induced seizures were induced by raising rectal temperature of 10-day-old rat pups with CD to 41°C in a heat chamber once a day for five days. On postnatal day 28, the animals were injected with a subconvulsive dose of pentylenetetrazole (PTZ) to assess seizure susceptibility. BBB permeability was evaluated functionally and ultrastructurally by determining extravasation of Evans blue (EB) and horseradish peroxidase (HRP) tracers, respectively. Subconvulsive dose of PTZ increased the mean Racine's scores of seizures from 3.42±1.4 to 4.3±0.4 in animals with CD exposed to hyperthermia-induced seizures. In these animals, extravasation of EB dye into the brain significantly increased upon subconvulsive PTZ treatment. Ultrastructurally, frequent vesicles containing HRP reaction products were observed in the cytoplasm of brain capillary endothelial cells in cerebral cortex and hippocampus of rats with CD subjected to hyperthermia-induced seizures and subsequently treated or untreated with PTZ. These results indicate that repeated hyperthermia-induced seizures make BBB leaky and increase seizure susceptibility in rats with CD which suggests that in this two-hit model, alterations in BBB permeability may facilitate epileptogenesis in the setting of repetitive hyperthermia-induced seizures.

Regulation of L-carnitine transport through the blood-brain barrier by protein kinase C activation

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L-carnitine (3-hydroxy-4-trimethylammoniobutyrate) is necessary in peripheral tissues for one of the essential catabolic processes – beta-oxidation of fatty acids, a process negligible in the adult brain, where carnitine has been demonstrated to be involved in acetylcholine synthesis, neurotransmitters metabolism and, especially in the form of acetylcarntine, in neuroprotection. The present study was focused on carnitine transport in an *in vitro* model of the blood-brain barrier (BBB), a co-culture of rat glial cells and bovine brain capillary endothelial cells grown on collagen coated microporous membrane. OCTN2 and ATB(0,+) were identified as transporters of carnitine across the BBB. OCTN2 is a sodium-dependent organic cation/carnitine transporter with a high-affinity for carnitine, while ATB(0,+) - a sodium- and chloride- dependent neutral and basic amino acid transporter, transports carnitine with a low affinity. The role of protein kinase C (PKC) in the regulation of this transport was analysed and carnitine transport was observed to be polarized and PKC dependent. Endothelial cells were observed to maintain a stable carnitine gradient, and transport was by twofold more efficient from basolateral than apical side. Accumulation of carnitine in endothelial cells is inhibited by PKC activation, but only when carnitine is transported from the apical side. We combined various methods, such as transmission electron microscopy, surface protein biotinylation and immunoprecipitation to determine OCTN2 and ATB(0,+) localization within the BBB and understand the way in which PKC influences carnitine transport.

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The molecular mechanism of blood brain barrier alterations in neurodegenerative diseases

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The endothelial cells tight junctions (TJs) are the most important structural component of the blood-brain barrier (BBB), so that any molecular alteration in the phosphorylation of TJs proteins, such as occludin and/or ZO-1 could be crucial in alterations of the BBB vascular permeability control. Regarding the specific structural and biochemical cellular components of BBB the astrocytes endfeet enveloping the vessels wall, have been proposed as an important determinant in the maintenance of the BBB integrity, through the secretion of soluble factors by the endothelial cells. Since, the astrocytes in BBB are able to control the water flux by aquaporin-4 (AQP4) as a specific water channel. The alterations in BBB can be led to a various complex events occurring in the progression of deferent neurodegenerative diseases, such as multiple sclerosis, Duchenne muscular dystrophy as well as mechanical injuries, septic encephalopathy and transient ischemia. In these diseases, the histopathological and microanatomical status are associated with BBB alterations and disruption along with vasogenic edema, swollen astrocyte endfeet as well as increase in microvascular permeability.

Inhibition of OGD-induced and astrocyte enhanced blood-brain barrier breakdown by specific receptor modulators

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In case of stroke, the reduction of blood supply results in a decrease of oxygen and glucose in local areas of the brain, which leads to blood-brain barrier (BBB) disruption and increased cerebrovascular permeability. The aims of the presented work were firstly to investigate the role of astrocytes during oxygen/glucose deprivation (OGD) induced BBB-breakdown, secondly to reveal receptor modulators which can block the OGD mediated BBB damage and thirdly to find regulated molecular targets and mechanisms. Mouse cell line cerebEND was used to model the BBB in vitro, rat glioma cell line C6 was applied as astrocyte model. Cells were exposed either in mono or co-culture set-ups to OGD (no glucose, 1% O2). Dependent on the assay format the OGD treatment was followed by an additional 20 hours lasting reoxygenation phase. Expression of tight junction (Cldn-1,-3,-5,-12, occludin, ZO-1, tricellulin) and ABC-transporter (abcb1, abcc4, abcg2) proteins was analyzed on the mRNA level by qPCR as well as on the protein level by western blotting or immunofluorescence microscopy. Physical and transporter barrier properties were determined by measurement of transendothelial electrical resistance, fluorescein permeability and uptake assays.
In summary, astrocytes modulated key properties of BBB cell line cerebEND during normoxia as well as after OGD-treatment. Application of four hours OGD changes expression and functionality of tight junction as well as transporter proteins significantly. Loss of barrier function due to OGD was significantly increased in the presence of astrocytes. BBB damage and molecular regulations were inhibitable by specific receptor modulators highlighting relevant signalling pathways potentially stabilizing BBB integrity during stroke.

MPO-derived 2-Chlorohexadecanal as Effector of BBB Function In Vitro and In Vivo

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High amounts of unsaturated lipids and high rates of oxygen utilization make the central nervous system (CNS) extremely vulnerable to oxidative stress. Therefore, generation of reactive radical species during chronic inflammation in the brain is a common feature of neurodegenerative disorders including Alzheimer's disease (AD), multiple sclerosis (MS), and Parkinson's disease (PD) which are accompanied by blood-brain barrier (BBB) dysfunction. Myeloperoxidase (MPO), a phagocyte key oxidant producing enzyme induced during inflammation was recently shown to be upregulated in brains of AD, MS, and PD patients. MPO activation leads to the formation of the potent oxidant hypochlorous acid (HOCl) that is able to modify nucleic acids, proteins and lipids.

HOCl readily targets the vinylether bond of the plasmalogen fraction, a quantitatively important and functionally indispensible lipid subclass in the CNS. This reaction generates chlorinated fatty aldehydes (e.g. 2-chlorohexadecanal (2-ClHDA)) and the remnant lysophospholipids. We could demonstrate plasmalogen modification and concomitant MPO-mediated generation of chlorinated fatty aldehydes in vitro and in vivo. Immunohistochemical and immunofluorescence studies revealed the cerebrovasculature as a major compartment of leukocyte activation and MPO release in a sepsis mouse model. 2-ClHDA impaired mitochondrial function in pBCECs and induced apoptotic cell death. In situ perfusion of rat brains with 2-ClHDA significantly impaired BBB function.

In summary the present study demonstrates 2-ClHDA formation in vitro and in vivo and indicates an extremely high lipotoxic potential of 2-ClHDA. These findings indicate that this chlorinated aldehyde might be a key player contributing to BBB dysfunction during neuroinflammation.

Analysis of quercetin derivatives in the cerebrospinal fluid of adult ewes

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As components of plant food products flavonoids, including quercetin, are consumed by humans in the amounts which may be significant from the physiological point of view. Since certain flavonoids have strong antioxidant properties therefore they may provide neuroprotection. It is known that quercetin is absorbed into the blood circulation system. However, it is not clear whether quercetin reach the brain in sufficient level and in a biologically active form to have any beneficial effects. Therefore the goal of this study was to determine the level of quercetin and its metabolites in the cerebrospinal fluid (CSF) after feeding animals with fodders rich in quercetin. Studies were performed on ovine model that allowed for repeated sampling of the CSF from the third ventricle of the brain and, in parallel, blood samples from the jugular vein and urine. Quercetin and its metabolites were measured by HPLC-MS/MS method. In all biological fluids, di- and mono-glucuronided derivatives of quercetin and isorhamnetin (methylated form of quercetin) as well as aglycone of quercetin and isorhamnetin were found. This indicates that biologically active forms of quercetin may reach the brain.

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Aquaporin-4 ablation impairs Blood Retinal Barrier permeability

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Maintenance and formation of the blood-retinal barrier (BRB) is required for correct vision. Impairment of this barrier contributes to the pathology of a broad number of retinal diseases. The endothelial water channel Aquaporin-1 (AQP1) is typically absent in the CNS at the level of endothelial cells forming the blood-brain barrier whereas Aquaporin-4 (AQP4) is strongly expressed at astrocyte endfeet and has an important role in mediating water flux at the blood-brain and CSF-brain barriers. Interestingly, AQP4 is the target of autoantibodies characteristic of Neuromyelitis Optica (NMO), a severe form of multiple sclerosis characterized by ambulation problems and impaired vision. In the present study we used CD1 and C57BL10 strains of AQP4 null mice to assess the role of AQP4 in maintenance of BRB. The expression of AQP4, together with the endothelial water channel AQP1, was analyzed by confocal microscopy in parallel with GFAP and CD31, glial and endothelial markers respectively. Results showed that AQP1 was not expressed in endothelial cells in both WT and AQP4 null mice, whereas AQP4 resulted strongly enriched in astrocyte endfeet and Müller cells in WT mice and absent in AQP4 null mice, as expected. The morphology and the density of blood vessels and microvessels, observed by CD31 staining, appeared similar between WT and AQP4 null mice. However, Evans Blue perfusion, performed to evaluate the possible impairment of BRB in AQP4 null mice, revealed extravasation and strong reduction of microvessels, indicating their higher fragility in the absence of glial AQP4. Even though additional studies are necessary to clarify the molecular mechanism linking AQP4 loss to BRB fragility, these results indicate that AQP4 plays a pivotal role in the maintenance of BRB properties and that alterations in AQP4 expression, due to anti-AQP4 autoantibodies, may cause impaired vision in NMO patients.

Plasticity and differentiation potential of porcine cerebral capillary endothelial cells in vitro

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In the present study we have focused on the differentiation potential of cloned porcine cerebral capillary endothelial cells (cEC), which were previously shown to reversibly display two different phenotypes (cobblestone-like phenotype, CS; spindle-shaped phenotype, SSP) depending on whether or not distinct growth factors are present in the culture medium [1]. To this end, CS and SSP cells were grown under various culture conditions including treatment with adipo-, chondro-, osteo-, and neuro-inductive culture media. Using immunostaining, morphologic inspection, and quantitative and qualitative PCR, alterations of gene- and protein expression as well as phenotypic variations and functional properties (e.g., migratory activity and tube formation) of CS and SSP type cells have been determined.

Results: As shown by immunohistochemistry both cell types are labeled by *Bandeiraea simplicifolia* lectin confirming their endothelial nature. On transcriptional level both cell types were shown to express Angpt 1/2, CD31, alphaSMA, CD13, MMP2 and markers for tight-junction proteins and molecular transporters of the BBB (Claudin1, ZO-2, Lat1, Mct1, IGF1R, TfRc and LEPR). These cells also express PPARγ2, a master gene regulating early adipogenesis. In contrast to SSP cells, CS type cells express VEGFR1, Glut1 and P-gp, whereas transcripts encoding PDGFR-β and osterix are found only in SSP type cells only. In a matrigel overlay assay CS type cells showed tube formation, while the SSP type cells did not rearrange after contact with matrigel. In adipo-inductive differentiation media only the CS type cells were able to differentiate into adipocytes, as evidenced by the presence of lipid droplets. On the other hand only SSP type cells were capable of performing ossification in culture in osteo-inductive medium. Cultivation in chondrogenic and neuroinductive media did not yield significant alterations so far. Based on our results we conclude that cerebral capillary endothelial cells exhibit a high differentiation potential. This is reflected by their ability to respond to adipo- and osteoinductive stimulation in vitro.

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Amyloid-beta 1-42 peptide-induced toxicity in the cells of the neurovascular unit: protection by docosahexaenoic acid

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Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β peptides (Aβ) as perivascular deposits and senile plaques in the brain. The intake of the polyunsaturated fatty acid docosahexaenoic acid (DHA) has been associated with decreased amyloid deposition and reduced risk in AD in several epidemiological trials; however the exact underlying molecular mechanism remains to be elucidated. The aim of the study was to test whether DHA can exert a direct protective effect on the elements of the neurovascular unit, such as neurons, glial cells, brain endothelial cells and pericytes, treated with Aβ42 (15 µM). A dose-dependent high cellular toxicity was found in viability assays in all cell types and on acute hippocampal slices after treatment with Aβ42 small oligomers prepared in situ from an isopeptide precursor. The cell morphology also changed dramatically. In brain endothelial cells damaged barrier function and increased para- and transcellular permeability. Elevation of the production of reactive oxygen radicals was observed in endothelial, glial cells and pericytes after peptide treatment. DHA (30 µM) significantly decreased the Aβ42-induced toxic effects in all cell types measured by viability assays, and protected the barrier integrity. These results indicate for the first time that DHA can protect not only neurons but also the other elements of the neurovascular unit from the toxic effects of Aβ42 and this double effect may be beneficial in AD.

Pathogenicity of Plasmodium falciparum field isolates: identification of the new immunological and therapeutic targets for severe malaria

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Plasmodium falciparum malaria is one of the life threatening diseases. The infection can abruptly progress to severe manifestations and cause about 781000 deaths each year. Several studies have implicated sequestration of P. falciparum-parasitizes red blood cells (PRBC) within endothelial cells from various organs including brain and lungs. Indeed, the knowledge of the nature of parasite ligands and their receptors from the host cells responsible of severe disease remains fundamental for specific pathway inhibitory drug design. Since several years we investigated the pathogenicity of P. falciparum field isolates. In different experiments, using a coculture model of human lung endothelial cells (HLEC)-P. falciparum, we found that as many as 20% of PRBC from the field collected in two local hospitals of Franceville, Gabon, induce HLEC apoptosis. Interestingly apoptosis was significantly associated with neurological sings (prostration coma) but there was no significant association between apoptosis and severe malaria clinical status as a whole (uncomplicated vs severe malaria). Analysis of whole transcriptome from apoptogenic vs non apoptogenic P. falciparum using DNA microarray technique revealed 59 genes overtranscribed in apoptogenic ones. These genes were mainly composed of enzymes and only 10 surface antigens. Among twelve (12) synthetic peptides with B and T cell epitopes designed from these antigens, four (4) are recognized by specific IgG and IgG1 from endemic subjects. On the other hand, when cocultures with contact are carried out we found that Fasudil (HA-1077) a Rho kinase inhibitor prevents endothelium apoptosis from all the P. falciparum isolates tested. Strategies of developing new immunological and/or therapeutic agents will be discussed.

Phloretin ameliorates MPO-mediated barrier dysfunction in brain microvascular endothelial cells

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Central nervous system (CNS) related diseases, such as brain stroke, Alzheimer's disease (AD) and multiple sclerosis (MS) are in general accompanied by blood-brain barrier (BBB) dysfunction. Within the last few years there is increasing evidence that myeloperoxidase (MPO) of phagocytes plays an important role in patho-physiological sequelae of neurodegenerative diseases with an inflammatory component. A unique property of MPO is the ability to form the potent oxidant hypochlorous acid (HOCl). Recently, we could demonstrate that oxidative damage of nervous tissue during acute neuroinflammation is accompanied by pronounced plasmalogen (a lipid class essential for normal CNS function) degradation and concomitant formation of highly lipotoxic chlorinated aldehydes, e.g. 2-chlorohexadecanal (2-ClHDA). The current study aimed to identify therapeutic useful compounds that are able to amend MPO-mediated BBB dysfunction. Results of the present study provide evidence that natural flavonoids e.g. phloretin have the ability to ameliorate toxicity of 2-ClHDA or HOCl and to restore barrier integrity of an in vitro BBB model. Further mechanistic explorations demonstrated that 2-chloro aldehydes and HOCl are selectively scavenged by phloretin and that the 2,4,6-trihydroxyacetophenone group represents the pharmacophore group. In summary, our results support the concept of dietary benefits to MPO-mediated chlorinative stress in the CNS and open potentially new pharmacotherapeutical strategies to interfere with aldehyde-mediated BBB dysfunction.

Affect of simulated high altitude environment exposed to the BBB permeability and sodium aescinate to protective role of BBB and anti-leakage mechanism under hypoxia

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Background and objectives: High altitude cerebral edema (HACE) is a kind of acute severe mountain sickness, it serious threatening to life-safety of person who rapidly entering altitude. But there is currently no effective treatment method.Therefore, observeing the treatment effects of aescine on HACE and its relative mechanism had great significant.
Methods: Adult male SD rats were randomly divided into plain control group (PC), plain drug group (PD), altitude hypoxic group (AH) and hypoxia treated group (HT), with 25 in each group. In AH and HT group, hypoxia treatment were in simulated altitude 8000m hypobaric chamber.The rats of HT and PD group was eceive intraperitoneal injection of sodium aescinate (5mg/kg.bw/day), continuous drug delivery 3 times. After experiment finished, brain water content, brain evans blue content, pathological and ultrastructural changes of brain and lanthanum nitrate particle distribution in brain were assayed, expression of occludin, ZO-1, claudin-5 gene and Occludin, NF-κB P65 protein in frontal and cortex were detected respectively.
Results: Compared with PC group, the content of brain water and evans blue were obviously increased in AH group.Hippocampal formation damaged seriously, pyramidal cells disarranged.Tight junctions widened, lanthanum nitrate particle leaked out from blood capillary and deposited into cortex, some of neurons and glial cells were swollen.The content of brain water and evans blue increase significantly, the expression levels of occludin, ZO-1 and claudin-5 mRNA were lower noticeably in AH group.In HT group had a light damaged, most of lanthanum nitrate particle located in endovascular while a little of which exuded to extracapillary and brain tissue space tissue showed mild edema. Occludin protein expression ascended, but contrary NF-κB P65 protein expression significant reduced.
Conclusions:Under acute hypoxia exposure, permeability of BBB increased significantly, sodium aescinate may be associated with up-regulation expression of occludin, ZO-1, claudin-5 and down- regulation expression of NF-κB P65, reduce BBB permeability, attenuate cerebral edema.

T cell diapedesis across the Blood-brain barrier endothelium: The inflammatory stimulus regulates the trans- versus the paracellular pathway

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Extravasation of T cells from the vasculature to the site of inflammation is a tightly regulated multi step process involving rolling, arrest, crawling and -finally- diapedesis across the endothelium. For diapedesis, T cells can take two passageways: The transcellular route or the paracellular route. During transcellular diapedesis a pore is formed through the cytoplasm of an endothelial cell – a process seemingly being initiated by invasive cell protrusions of the T cell. Paracellular diapedesis is thought to occur through a zipper like process in which inter-endothelial protein-protein interactions are replaced by endothelial-T cell protein-protein interactions. In vitro studies have demonstrated that T cell diapedesis proceeds along both pathways and varies according to the type of endothelium. The blood brain barrier (BBB) is a highly specialized endothelium protecting the central nervous system (CNS) from harmful substances in the blood stream through an elaborate network of tight junctions limiting the paracellular pathway. Therefore, we hypothesized that the extreme tightness of BBB endothelium translates into transcellular over paracellular diapedesis of T cells. To determine the route of T cell diapedesis across the BBB we isolated primary mouse brain microvascular endothelial cells (pMBMECs) from transgenic mice expressing VE-cadherin-GFP under control of the endogenous genetic VE-cadherin locus and studied the pathway of T cell diapedesis with live cell imaging microscopy in an in vitro flow chamber set up. Because under inflammatory conditions high numbers of encephalitogenic T cells extravasate across the BBB and critically contribute to inflammatory diseases of the CNS, we compared the effect of different cytokine for the pathway of diapedesis. To analyse the influence of endothelial ICAM-1 on the pathway of T cell diapedesis, we have cross-bred VE-cadherin-GFP transgenic mice with ICAM-1 deficient mice. Currently, pMBMECs isolated from these animals are used to test, whether endothelial ICAM-1 is essential for one or the other pathway of T cell diapedesis across BBB endothelial cells.

Computational approach to the prediction of blood-brain barrier permeability using density functional theory

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The usefulness of the quantum chemical descriptors, calculated at the level of the DFT theoriy using 6_31G* basis set for QSAR study of anti_viral Nucleoside Analogues drugs was examined. Delivery of anti_viral agents into the central nervous system (CNS) is clinically important. Nocleoside analogues are a major source of clinically used antiviral agents. The QSAR model developed contributed to a mechanistic understanding of the investigated biological effects. Biological activities contain the logarithm of the ratio of the steady_state concentration of a compound in the brain to in the blood, logBB.
A multi_parametric equation containing maximum four descriptors at DFT method with good statistical qualities (*R*max = 0.996, = 0.991 at B3LYP/6_31G*) was obtained by Multiple Linear Regression (MLR) using stepwise method.

Effect of hypoxia and of caprate on claudins in isolated murine brain capillaries

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Claudins are expressed at the tight junctions (TJ) in the brain. Claudin-5 (Cld5) limits the diffusion of small, drug-like hydrophilic molecules through the blood-brain barrier. Occludin is assumed to be of regulatory relevance under pathological conditions, such as oxidative stress. Both are scaffolded by the recruiting protein of the TJ Z*onula occludens* protein 1 (ZO-1). Here, we study the effect of hypoxia and of the drug enhancer caprate on the TJ proteins of the blood-brain barrier. For this purpose, cerebral capillaries were isolated from mice and incubated under hypoxic or normoxic conditions, and with Na-caprate, respectively. Immunofluorescence analyses were done with confocal laser scanning microscopy.
The TJ areas of the capillaries were identified with the junctional marker ZO-1 and were visible as one or two linear patterns through the capillaries where the endothelial cells attach each other (cell-cell contacts). ZO-1 did not change its expression and localization at all instances. Therefore, ZO-1 could be used as reference and marker protein of the TJ. Thus, the localization and expression of Cld5 was detected and checked in respect to the colocalization with ZO-1. Comparing normoxic and hypoxic conditions, ZO-1 and Cld5 expression patterns remained unchanged in capillaries, which indicate that hypoxic conditions did not alter the general TJ protein pattern in the blood-brain barrier during 5 h of hypoxia. Caprate, a clinically used absorption enhancer for pharmacologically active agents is known to open the paracellular space by acting on the TJ with an unspecific mechanism. Its action on Cld5 is not understood so far. Under normoxic conditions caprate reversibly removed Cld5 from the cell contacts without affecting ZO-1. Thus, it was shown that Cld5 is a potential pharmacological target to improve drug delivery to tissues sealed by Cld5-dependent cell barriers which could be used to bypath the blood-brain barrier under hypoxic condiditions, for instance, to treat ischemic brain edema.

Central inflammatory markers in response to surgical stress and in relationship to personality.

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Background: Accumulating evidence supports an intricate relationship betweeninflammation, stress and personalityThere are few studies regarding personality, stress and the immune system in non-psychiatric populations. Objective: We wanted to investigate how this relationship is mirrored in the levels of inflammatory markers in cerebrospinal fluid (CSF). Methods: 35 patients undergoing knee arthroplastics had CSF samples drawn before, three hours after and the morning after surgery. Cytokine concentrations were assessed at all three points. Before surgery, subjects filled in two personality assessment questionnaires: Temperament and Character Inventory (TCI) and Karolinska Scales of Personality (KSP). Results: Serum IL-8 and IL-10 increased during and after surgery. Serum TNF-alpha decreased significantly after surgery. CSF IL-2, IL-8, IL-10, IL-13 and TNF increased significantly during and after surgery. CSF IL-5 increased significantly aftersurgery. Correlations were found between low CSF IL-10, inhibited aggression and social anxiety, and between high CSF IL-10 and verbal aggression. CSF IL-8 was negatively correlated with Conscience. Serum IFN-□□and IL-10 correlated positively with Guilt and negatively with self-directedness, and, for IFN-□, Goal-orientation. There was a negative correlation between serum TNF and Helpfulness. Conclusion: There seem to be few other studies assessing central inflammatory markers in response to peripheral surgery. We found marked increases in brain cytokines that differed from their peripheral fluctuations. In addition, several of the cytokines were correlated with destructive personality traits at basline. These relationships between aspects of personality and levels of different inflammatory markers are worthy of further investigation.

Investigating claudin-3 and claudin-5 functions at the Blood-Brain Barrier

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The presence of tight junction (TJ) proteins between endothelial cells forming the blood-brain barrier (BBB) is responsible for its barrier function in restricting passage of blood borne molecules through the paracellular cleft between adjacent endothelial cells. Members of the claudin family, such as claudin-5 and claudin-3, seem to represent the major TJ proteins involved in barrier properties, although their specific functions at the BBB have never been fully addressed.
To elucidate the precise role of claudins in BBB functions, we cultured primary mouse brain microvascular endothelial cells (pMBMECs) from claudin-$3^{-/-}$ and claudin-$5^{+/-}$ mice (as claudin-$5^{-/-}$ die shortly after birth (Nitta et al., *J.Cell Biol.*, 161:653, 2003)). Immunofluorescence staining for tight junction components revealed no difference in expression and TJ localization of occludin and claudin-5 in claudin-$3^{-/-}$ BBB endothelia. Surprisingly, the claudin-3 antibody used (Invitrogen), which did not cross-react with claudin-1 or claudin-5, also showed a positive staining in claudin-$3^{-/-}$ pMBMECs, indicating a cross-reactivity of this antibody with another junctional molecule. No difference in the expression and the TJ localization of occludin and claudin-3 could be observed in claudin-$5^{+/-}$ BBB endothelial cells, however preliminary observation of claudin-5 immunostaining seems less intense at TJs in claudin-$5^{+/-}$ endothelial cells compared to wild-type. Nonetheless no significant differences in the paracellular integrity of the BBB endothelium, following permeability assays to 3KDa dextran and TEER measurement, were visible in claudin-$3^{-/-}$ and claudin-$5^{+/-}$ conditions compared to wild type. In vivo, neither Evans Blue bound to albumin (67.5 KDa), nor Hoechst dye (530 Da) diffused into the brain parenchyma after their intra-venous injection into claudin-$3^{-/-}$ or claudin-$5^{+/-}$ mice indicating no change in BBB permeability for components - 530 Da in both claudin-$3^{-/-}$ and claudin-$5^{+/-}$ mice compared to wild type. Thus, the specific function of individual claudin expressed at the BBB still needs further characterization especially during neuroinflammation where loss of claudin-3 expression from the BBB TJs correlates to impaired BBB integrity, as well as during embryogenesis, where increased claudin-3 expression has been linked to barrier maturation regulated through Wnt/beta-catenin signaling.

Software to generate Computer Aided Design (CAD) of brain capillary network for Finite Element Model simulation

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In order to facilitate the preparation and execution of simulation of blood flow and diffusion through the membrane in brain capillary networks a software has been developed as a macro that accelerates the generation of the starting geometry. By designing the base geometry and input the parameters considered most relevant a code has been generated that saves time and effort when drawing the desired structure. The steps followed in developing the program are based on the choice of a model capillary network from which the user selects the parameters of interest. Capillaries were drawn first following different stable strategies. Macros were recorded for different stages of drawing and the associated code was analysed in order to be a parametric design. A final version was developed based on Microsoft Visual Basic 2010 Express and CATIA®, respectively. The initial design considered cylinders of around 6.0 µm in diameter which are divided into branches to reach diameters of few micrometers. With the use of this software in just few clicks a new CAD is generated to study the influence of a relevant parameter such as initial radius, number of branches, … . The CAD data is automatically transferred to simulate Finite Element Model simulations with Computer Fluid Dynamics techniques and also to generate prototypes with additive manufacturing techniques.

Up regulation of different blood brain barrier in vitro models by astroglia

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There is a growing interest to minimize the use of animal experimentation mainly due to ethical issues and high experimental cost. On the other hand, the design and discovery of drugs that can readily cross the blood brain barrier (BBB) is a major bottleneck in the development of drugs targeting the Central Nervous System. Therefore the development of in vitro BBB models that preserve in vivo transporter functions to assess drug toxicity, permeability and safety at the earliest stages of drug discovery is of utmost importance for neuro-medicine. The aim of the present study was to evaluate the role of astrocytes on the modulation of paracellular permeability and morphogenesis in different BBB cell culture-based models. Three cell lines: human intestinal cell line (Caco-2), the kidney cells (MDCKII) and the brain endothelial cells (bEnd.3), were cultured alone(1); with astrocytic factors(2); on conditioned medium(3) and co-cultured (4) with rat primary astrocytes. The development of the different in vitro BBB models was monitored by the measurement of the transendothelial electrical resistance and enzymatic activities as γ-glutamyl transpeptidase activity and alkaline phosphatase activity. Moreover, Lucifer Yellow, Dextrans 10, 40 and 70 permeability assays and tight junction citometry studies were carried out to detect functional differences on the BBB models. The results showed that the presence of astrocytes in the co-cultures and the use of astrocytic factors and conditioned medium enhances the properties of the BBB models. However, the different culture conditions work at different ways. Adhesion, enzyme activity and permeability properties of the BBB models change depending on the type of the endothelial cell and culture form, noticing a poor correlation between the three in vitro models. Future efforts should be directed towards improving existing models.

In vitro evidence for the brain glutamate efflux hypothesis; brain endothelial cells co-cultured with astrocytes display a polarized brain-to-blood transport of glutamate

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The aim of this study was to investigate the role of the blood-brain barrier endothelium in brain L-glutamate homeostasis. EAAT mRNA and protein expression was investigated in an electrically tight bovine endothelial/rat astrocyte blood-brain barrier co-culture model, and expression differences were examined in mono-cultures as well as contact and non-contact co-cultures. Furthermore abluminal uptake of ^{3}H-L-glutamate was characterized in non-contact co-cultures and transendothelial transport and accumulation studies of ^{3}H-L-glutamate, ^{3}H-L-aspartate and ^{3}H-D-aspartate in contact co-cultures.
Expression of EAAT-1 was consistent on both mRNA and protein level in all culture setups as well as in freshly isolated capillaries, whereas EAAT-2 and -3 were only present in the co-culture models. However, mRNA data revealed that expression levels of EAAT-2 and -3 were low, also in the co-culture model. Abluminal uptake of glutamate caused an intracellular concentration 20 times higher than the donor concentration within 5 minutes. The uptake was inhibited with the EAAT inhibitor, DL- *threo*-ß-benzyloxyaspartate, down to 25 – 30 % of control and further displayed Michaelis Menten kinetics with V_{max} of 45 ± 7 pmol cm^{-2} min^{-1} and a K_M of 23 ± 15 µM. The transcellular transport studies were performed in the contact co-cultures, which displayed higher paracellular tightness. After six days in culture, the endothelium displayed transendothelial resistance values of 1014 ± 70 Ω cm^2, and ^{14}C-D-mannitol permeability values of 0.88 ± 0.13 × 10^{-6} cm s^{-1}. Unidirectional flux studies showed that L-aspartate and L-glutamate, but not D-aspartate, displayed polarized transport in the brain-to-blood direction, however all three amino acids accumulated in the co-cultures when applied from the abluminal side. The transcellular transport kinetics were characterized with a K_m of 138 ± 49 µM and J_{max} of 28 ± 3.1 pmol min^{-1} cm^{-2} for L-glutamate. Overall, the findings suggest that the blood-brain barrier itself may participate in regulating brain L-glutamate concentrations through a combination of EAAT-mediated uptake at the abluminal membrane and an unknown transporter-mediated efflux across the luminal membrane.

Signal transduction to human central nervous system (CNS) becomes modulated with blood-brain barriers, uncovered with the Marburg cerebrospinal fluid (CSF) model

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Signals (transmitters, hormones, specific proteins) to human CNS become modulated with 2 blood-brain barriers (bbb), evaluated with Marburg CSF model: bbb localized in all brain capillaries (tight to blood proteins (BP)); blood-CSF-barrier (bCSFb) in plexus choroidei where suboccipital (SOP) CSF is filtered from blood to 200 mg/l proteins through the molecular sieve of tight junctions. SOP-CSF enters spinal canal to flow off into valve-less lymph vessels; latent equilibrium between lymph and CSF pressures allows adding BP into SOP-CSF, elevating proteins up to 450 mg/l in lumbar CSF e.g. with 0.3 ml lymph. Published data from patients` lumbar CSF and blood serum, showing reference values, were evaluated to verify the postulates of Marburg CSF model.
Transmitter signals: Small transmitters <200 D pass the barriers in nearly 1 nMol-per-1 nMol ratios (Q): noradrenalin, dopamine, γ-aminobutyric acid; serotonin levels are higher in blood, adrenalin ones in CSF indicating more synthesis in body / in CNS; as it is found with bigger Met-enkephalin (573 D), ß-endorphin (25 kDa) revealing no equilibrium through the barriers.
Hormone signals: Hypophysis hormone ACTH (4.5 kDa) is secreted into CSF > blood elevating QACTH > 1.0. This is not the case with bigger prolactin, thyreotropin, LH, FSH, growth hormones. Low H20-soluble estradiol, testosterone, L-thyroxine (T4), cortisol need specific binding proteins, thus bCSFb control their feedback into CNS.
Signals of specific proteins: Transfer of β2-microglobulin (11.8 kDa) and α1-microglobulin (33.3 kDa), influencing immune defence, appears to be not restricted into CNS; whereas transport of Fe / Cu with transferrin (90 kDa) / caeruloplasmin (151 kDa) is, indicating a restriction similarly as albumin (66.3 kDa) / IgG (150 kDa).
Conclusion: bCSFb controls the signals from blood to CNS according to molecule seize being not altered by small lumbar blood reflux; thus confirming the postulates of Marburg CSF model.

Differential response to stress induced analgesia in two mouse strains: a role for the BBB?

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Located at the interface of the CNS and blood circulation the BBB is implicated in the pathogenesis of a considerable number of neurological disorders. By limiting the transport of compounds with analgesic effects the BBB could play a role in pain perception as well. Here we invesigated the response to different analgesics in two mouse lines: one with high sensitivity (HA) and the other with low sensitivity (LA) to stress induced analgesia. In response to administration of morphine which is able to cross the BBB we found no significant differences in the MPE (maximal possible effect) of the analgesic. However, when we used the biphalin analogue AA2016 we obtained a low MPE in LA animals and an effect comparable to morfine in HA animals. Endomorphine, an endogenous opioid, had a considerably lower analgesic effect in LA animals than in HA animals. This prompted us to investigate the BBB in the two mouse lines. Electron microscopic analysis revealed that in HA animals the ultrastructure of capillaries forming the BBB is altered: a thin basement membrane, unusually thin endothelial cells and low number of tight junctions were observed. In addition fenestrations and swollen astrocytic processes could also be detected. In contrast, capillaries of LA animals showed normal morphology. Analysis of the expression of tight junction proteins in capillaries revealed that the amount of occludin and claudin-5 was lower in HA animals compared to LA animals indicating a disfunction of the interendothelial contacts. Our results indicate that an altered BBB stands at the basis of the differences to stress response in the two mouse lines: endogenous opioid peptides released by stress in the perifery can easier penetrate into the CNS in HA animals and induce central analgesia. Furthermore, our model may serve as a natural model of increased BBB permeability.

The role of beta 1 integrins in a novel *in vitro* blood-brain barrier model

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The blood-brain barrier is necessary to provide an optimal chemical environment for cerebral function. It consists of specialized endothelial cells that interact through interendothelial tight junctions and form a barrier with low permeability. Thereby the infiltration of lymphocytes into central nervous system is limited. Pathological conditions, e.g. chronic-inflammatory diseases and viral infections, induce blood-brain barrier breakdown which facilitates the accumulation of immune cells in the brain.
Using the human endothelial cell line Transformed Human Brain Microvascular Endothelial Cells (THBMEC) we could establish an *in vitro* blood-brain barrier model that is characterised by a transendothelial electrical resistance (TEER) of 250 Ohm×cm^2 and a permeability coefficient of 1×10^{-6} cm/s. Stimulation with interleukin-1 beta (IL-1 beta) leads to a significant increase of inflammatory markers such as VCAM-1 and ICAM-1. In addition, significant reduction in TEER, increase in permeability as well as enlarged numbers of transmigrated lymphocytes could be observed in this system. Other inflammatory cytokines, such as TNF alpha, IFN gamma, IL-6, IL-17 and IL-22, had no significant effect on these parameters in this *in vitro* blood-brain barrier model.
We used this model for investigating the influence of beta 1 integrins, especially alpha5 beta1 integrin and alpha6 beta1 integrin, as well as the anti-inflammatory compound Ino-C2-PAF, on the blood-brain barrier integrity and transendothelial migration of lymphocytes under physiological and inflammatory conditions.

Tight Junctions Form Barriers in the Retinal Nerve Fiber Layer of Teleost Fish

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The interface between the mammalian retina and the vitreous humor is formed by a basal lamina and the endfeet of Müller cells which express a high density of water and potassium channels but do not form a tight barrier. In the vertebrate retina, blood supply is provided by intraretinal blood vessels and the choroid. However in many teleost fish such as cichlids, intraretinal vessels are missing. In the course of investigating the vitreous-retinal interface in the fish eye of Astatotilapia burtoni we discovered conspicuous tight junctions in the retinal nerve fiber layer by freeze fracture electron microscopy. These tight junctions formed branching strands between myelin-like wrappings of ganglion cell axons. However, these junctions were morphologically different from known myelin tight junction strands. Moreover, an elaborate meshwork of tight junction strands were found on large membrane faces belonging to glial cells in the nerve fiber layer. Using immunocytochemistry, the nerve fiber layer was indeed positive for the adaptor protein ZO-1. In addition, we detected immunoreactivity to antibodies directed against the mammalian claudin-1.
Currently, we are testing to what extent these junctions form a barrier in the nerve fiber layer separating the vitreous from the neural retina. In this context it is of interest that in the CNS of teleost fish, neurons and glial cells are continuously added from distinct proliferation zones throughout life. This implies that neuronal signaling occurs in close proximity to locations where processes of cell growth and proliferation have to be regulated. In fish, retinal tissue is added to the existing retina from a peripheral growth zone where the nerve fiber layer is missing or extremely thin. We speculate that the retinal periphery might therefore have access to growth promoting substances derived from ciliary blood vessels whereas in the central retina this might be prevented by the described tight junctions.

Circulating tight-junction proteins as predictors of clinically evident hemorrhagic transformation in ischemic stroke patients

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Severe secondary hemorrhagic transformation (HT) after ischemic stroke can lead to unfavorable outcomes. Breakdown of the blood-brain barrier (BBB) is associated with an increased risk of developing an HT and particularly with secondary HT in ischemic stroke patients. Tight junction (TJ) proteins are important cell adhesion components that are responsible for maintaining the BBB integrity. The aim of the study was to evaluate the significance of circulating TJ proteins as predictors of HT in ischemic stroke patients.
Material and Methods: The study included 458 consecutive ischemic stroke patients not treated with thrombolytic drugs. Among the studied subjects 7.2% had clinically evident HT. Serum levels of S100B protein, neuron - specific enolase (NSE)), TJ proteins (occludin (OCLN), claudin 5 (CLDN5), zonula occludens 1 (ZO1)), matrix metalloproteinase 9 and vascular endothelial growth factor (VEGF)) were estimated upon admission to the emergency room by means of ELISA. A clinical deterioration caused by HT (cdHT) was defined as an increase of -4 points in the National Institutes of Health Stroke Scale score in combination with a visible HT on a CT scan performed immediately after the onset of new neurological symptoms.
Results: The concentrations of OCLN, S100B and the CLDN5/ZO1 ratio were higher, and VEGF - lower in patients with cdHT comparing to those without cdHT. CLDN5 levels also correlated with cdHT occurrence when estimated within 3 hours of stroke onset. The levels of circulating TJ molecules correlated with the concentration of S100B.
Conclusions: The levels of circulating TJ proteins, S100B, VEGF as well as Claudin5/ZO1 ratio is an effective way to screen for clinical deterioration caused by HT in ischemic stroke patients. It may be applicated both within and after the intravenous thrombolysis time window.

Determinants contributing to claudin barrier and ion channel formation

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Proteins of the claudin family determine the characteristics of tight junctions (TJ), the most apical cell-cell contacts between epithelial and endothelial cells. Some claudins (Cld), e.g. Cld-1, -3, -4, and -8 seal the paracellular cleft, enabling the fence function of TJ, while other claudin molecules, such as Cld-2, -10, -16, and -17, form paracellular channels permeable for ions. Claudins have four transmembranal domains and two extracellular loops (ECL1 and -2). Pore-forming properties of claudins are likely defined by residues of their ECL1. Up to now, the detailed structure and mechanism of pore formation remain unclear. We replaced ECL1 residues of Cld-1, a sealing claudin present in blood-cerebrospinal fluid barrier, by corresponding residues of Cld-2, a cation channel-forming claudin also present in blood-cerebrospinal fluid barrier, to identify new determinants responsible for sealing and/or pore formation. After creation of MDCK cell lines stably overexpressing FLAG-Cld-1 wild-type and mutants thereof, the subcellular localization of transfected FLAG-Cld-1 constructs was analysed and expression of overexpressed claudins as well as of endogenous Cld-1, -4, -3 or -7 was verified. The obtained clones were characterized by transepithelial resistance (TER) and ion permeability measurements. We found that E48K and S53E substitutions in human Cld-1 strongly reduced TER and increased permeability for Na^+ and Cl^-. In contrast, K65D, D68S, and other single substitutions showed no significant change of TER and permeability for Na^+ and Cl^-. Double substitution S53E/K65D did not change TER and ion permeability, whereas S53E/D68S decreased TER, albeit weaker than S53E. The ratio of permeabilities for Na^+ and Cl^- revealed no clear charge specificity of the pore induced by S53E or S53E/D68S in Cld-1, suggesting that primarily S53 and potentially D68 in Cld-1 are involved in sealing of the paracellular cleft and that charge-unselective pores may be induced substituting S53E. We provide novel molecular determinants as parts of the puzzle for solving different molecular mechanisms distinguishing sealing and pore formation of claudins, both relevant for blood-brain and blood-cerebrospinal fluid barriers.

Influence of Valproic Acid on Temozolomide transport through Blood-Brain Barrier in vitro model

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The blood-brain barrier is a selective membrane which separates the circulation system from the central nervous system (CNS). The main function of the blood-brain barrier is to prevent the passage of any potentially toxic and harmful substances in the CNS. In the experiment the Madin-Darby Canine Kidney (MDCK) cells have been utilized as a form of epithelial cells to determine the transepithelial transport.The MDCK cells have been employed as a tool for selective membrane permeability screening as these cells display rapid membrane permeability. The MDCK cells provided a selective barrier for transport of the antineoplastic drug Temozolomide along with the antiepileptic drug, Valproic acid (VPA). Temozolomide (TMZ) has been extensively used in its effective treatment against malignant gliomas of the brain and can be applied in pharmacotherapy with the combination of VPA due to symptoms of convulsions.
In the experiment the most accelerated permeability speed of TMZ through the MDCK monolayer was 2.82×10^{-6} cm/s with 50 µM of TMZ concentration. The transport of TMZ in the presence of VPA was determined through the applied MDCK cell monolayer and the fastest permeability speed was 2.21 cm/s, demonstrated during highest concentration of 100 µM VPA. In the co-cultured endothelial monolayer with astrocyte cells the co-administration of TMZ with higher concentration of 100 µM VPA displayed the most rapid speed of permeability of transport to be 4.8 cm/s.

Quantitative assessment of blood-brain barrier dysfunction and cell damage *in vivo* after cortical photothrombosis

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Stroke is one of the leading causes of death and morbidity worldwide and its treatment remains a clinically unmet challenge. The ischemic brain is typically characterized by an ischemic core and a peri-ischemic zone prone to undergo cell damage. The peri-ischemic brain may either recover or deteriorate, leading to secondary stroke progression involving complications, e.g. hemorrhagic transformation, delayed cognitive decline and epileptogenesis. Recently, blood-brain barrier (BBB) dysfunction has been indicated as a potential common denominator for post-stroke complications. However, to date methods to quantify BBB permeability and cell damage in-vivo are limited. Here we introduce a novel imaging technique designed to investigate the spatial and temporal correlation of BBB dysfunction and cell damage in-vivo in the peri-ischemic region of rose bengal-induced neocortical photothrombosis.
BBB permeability and cell damage were assessed in rats through pial surface imaging (open cranial window method) following the peripheral injection of the tracer molecules fluorescein sodium salt (BBB permeability) and propidium iodide (PI, cell damage). We demonstrate that BBB permeability increases most prominently in the perfused region surrounding the ischemic core within minutes after thrombus formation. The region of augmented vascular permeability gradually expands and is associated with increasing uptake of PI into cells, suggesting progressive cellular damage within the peri-ischemic brain. Simultaneous imaging of vascular permeability and cellular damage together with electrophysiological recordings is expected to reveal the sequence of events leading to progressive damage in the peri-ischemic region including the causative role of vascular permeability.

Exploring the Brainpeps database

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Since the discovery that peptides can cross the blood-brain barrier (BBB), doors have been opened to new therapeutics for CNS diseases and pain management. Recently, we have constructed the Brainpeps database (*brainpeps.ugent.be*) to give an overview of the available BBB transport data of peptides, which are scattered in the literature [1]. One possible application of the Brainpeps database is the study of structure-property relationships (QSPRs). Before peptides can be used as drugs, their impurity profile needs to be examined as part of the International Conference on Harmonization (ICH) risk assessment of peptide drugs. Compared to small molecules, no *in-silico* predictive programs are available for toxicity screening of the different peptide impurities towards passing the BBB. To predict the BBB-behaviour of peptides as well as their impurities, we explored the Brainpeps database. During this presentation, the first results of the modelling experiments are presented. Our starting hypothesis is that the interactions of peptides at the blood-brain barrier are comparable with those of peptides in HPLC systems. Therefore, we determined the retention characteristics on different fused-core HPLC systems of a set of model peptides selected from the Brainpeps database and explored the relationship between the chromatographic characteristics and their BBB-influx properties [2]. In conclusion, using the Brainpeps database and experimental HPLC data, a first step towards *in-silico* profiling of peptides, including their impurities, at the blood-brain barrier level is taken. More chromatographic analyses of BBB peptides and harmonization on testing the BBB transport of peptides are future challenges to validate and unify this model.

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Transmigration of polymorphonuclear neutrophils and monocytes through the human blood-cerebrospinal-fluid barrier after bacterial infection in vitro

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Bacterial meningitis is a severe disease in humans. To cause a meningitis bacteria have to cross either the endothelial cells of the blood-brain barrier or the choroid plexus epithelial cells of the blood-cerebrospinal fluid (CSF) barrier (BCSFB). After bacterial invasion leukocytes are recruited into the CNS secreting mediators such as IL-8, causing massive inflammation. The aim of this project is to identify the mechanisms of transmigration of PMN and monocytes during the transepithelial transmigration (TEM) process.
Using inverted transwell filter systems of human choroid plexus papilloma cells (HIBCPP), we studied PMN and monocyte recruitment over infected HIBCPP mimicking the BCSFB. Within this model we determined TEM rates of the immune cells, their migration route by immunoflourescence, electron microscopy and secretion of cytokine by cytokine bead array.
PMN show a significantly increased level of TEM after infection with wild-type *Neisseria meinigitidis* (MC58), but not with its unencapsulated mutant. Paracellular permeability and transepithelial electrical resistance confirmed an intact cell layer displaying barrier characteristics during TEM. With help of electron microscopical-images and immunofluorescence we observed para- as well as transcellular migrating PMN. Further analysis of secreted cytokines/chemokines showed increased levels of GRO/IL6/IL8/IL1-/IL1-/IL1ra/MIP1b/MCP-1/TNF-. In contrast to PMN transmigration we found a significantly decreased monocyte transmigration after infection of HIBCPP.
Our findings provide evidence that PMN can migrate para- and transcellular over the BCSFB after *N. meningitidis* infection. Chemokines such as GRO may be involved in this process. However, a possible regulation of cytokines, chemokines or cell adhesion molecules after infection may be responsible for the decreased TEM rate of monocytes.

Sodium caprate transiently opens claudin-5-containing barriers at tight junctions of epithelial and endothelial cells

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The blood brain barrier (BBB) limits the therapy of many CNS diseases and the BBB can be involved in brain disorders. Claudin-5 (Cld5), in the tight junction (TJ) of the BBB, reduces the paracellular permeation of small molecules (<800 Da, Nitta *et al*., 2003). Cld5 oligomerize in the plasma membrane of the same cell (*cis*-interaction) and between plasma membranes of adjacent cells (*trans*-interaction). In this context, we are searching for molecules which temporally open the BBB by targeting Cld5, to improve drug delivery into the brain. Sodium-caprate (C10) is known to open TJ and it is clinically established. However, the action on claudin-claudin interactions and on TJ molecular components was not fully understood.
C10 reversibly reduced Cld5 *trans*-interaction in living TJ-free HEK-293 cells stably transfected with Cld5-YFP in a concentration- and time-dependent manner. To further clarify the mechanism by which C10 disrupts Cld5 *trans*-interactions, we employed MDCK-II cells stably transfected with N-terminal Flag-tagged Cld5 allowing the investigation of the complete TJ molecular machinery. C10 decreased the membranous Cld5 and F-actin content, which resulted in increased permeability of the small molecule lucifer yellow (400 Da). Interestingly, *zonula occludens* protein 1 (ZO-1) which links the actin cytoskeleton to claudins was mainly resistant against C10. Similarly as in epithelial cells, endogenous Cld5 in the membrane of brain endothelia cells was displaced together with F-actin, whereas ZO-1 remained unaffected.
In conclusion, we showed that C10 transiently opens the paracellular space, reducing the intercellular Cld5-Cld5 interactions and F-actin at the peri-junctional region of endothelial and epithelial cells. The study further expands the applicability of caprate as drug enhancer on claudin-5 containing tissue barriers.

Nitta *et al*., *J.Cell Biol., 161*:653, 2003

cCPE as a potential tool to affect claudins, present in BBB

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The C-terminal domain of *Clostridium perfringens* enterotoxin (cCPE) is a promising modulator of tight junctions. cCPE binds to the second extracellular loop (ECL2) of a subset of claudins (Cld), e.g. Cld-3, -4, but not to Cld-5 and only with weak affinity to Cld-1. Recently we could show that cCPE exhibits different binding modes towards Cld-3 and Cld-4 and were able to utilize these findings to create cCPE constructs which interact subtype specific preferentially either with Cld-3 or with Cld-4. In addition, we established a molecular interaction model of cCPE bound to the ECL2 of Cld-3 and Cld-4. Based on these findings we now studied why cCPE is only binding with weak affinity to Cld-1 and shows no binding to Cld-5. Cld-3 and Cld-4 share a common sequence-motif in the turn region of the ECL2 (NP L/VVA/P) which mediates the cCPE binding, whilst Cld-1 has the sequence DPLTP and Cld-5 the corresponding sequence DPTVP at these positions. Exchange of the residues D150 and T153 in Cld-1 and D149 and T151 in Cld-5 to the corresponding residues in Cld-3 leads to Cld-1 and -5 constructs with an enhanced cCPE-binding. These results offer first insights on strategies for the creation of cCPE constructs which target Cld-1 and/or Cld-5 specifically.

Mono-, double and triple co-culture models of the blood-brain barrier: a gene-array study

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A great number of cell culture–based blood–brain barrier models have been developed in the last 30 years. Endothelial cells were used as monolayers in the first studies. Since brain endothelial cells lose easily their specific characteristics in culture monolayers were replaced by co-culture systems using glial cells and recently by triple co-culture settings with pericytes. The importance of crosstalk between the cells of the neurovascular unit in the induction of BBB properties has been investigated in a gene array study. To compare the relative mRNA expression levels of selected tight junction proteins, transporters and metabolic enzymes TaqMan assays were performed on rat primary brain endothelial cells of mono- and co-culture BBB models. Among the barrier tightening claudins the expression of Cldn-3, Cldn-5 and Cldn-11 mRNA was higher in brain endothelial cells co-cultured with astroglia and pericytes compared with monocultures in agreement with our previous data on barrier tightness. From the family of solute carrier transporters responsible for brain nutrition higher amount of glucose transporter-1, -3 (Glut-1, -3) and monocarboxylic acid transporter-1, -3 (Mct-1 -3) mRNA was detected in co-culture models. Glutamate transporters Eaat1 and Eaat3 were also expressed at high levels in the triple model. The gene expression level of efflux pumps P-glycoprotein, breast cancer resistance protein (Bcrp) and multidrug resistance proteins Mrp-1, -3, -4, -5 were elevated by glia and pericytes in brain endothelial cells. From the examined phase I and II metabolic enzymes higher level of expression was detected for Cyp2s1 and Cyp2u1, glutathione S-transferase and sulfotransferase (Sult1a1) in co-cultures. In conclusion culture of endothelial cells in the presence of both glial cells and pericytes induced the expression of genes contributing to BBB characteristics.

Blood-brain barrier changes in ornithine-induced acute pancreatitis model

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Acute pancreatitis is a sudden inflammatory disorder of the pancreas caused by rapid inappropriate activation of pancreatic enzymes destroying the exocrin part of the organ. Pancreatic encephalopathy, an early, uncommon complication of the disease results in high mortality rate. Elevated permeability of the blood-brain barrier (BBB) was demonstrated in taurocholate-induced experimental models of severe acute pancreatitis in our previous study and in the literature. A novel, noninvasive, reproducible model of severe acute pancreatitis was introduced by the intraperitoneal administration of basic aliphatic amino acid L-ornithine in rats (1). The aim of this study was to test the changes in BBB permeability and morphology in rats with pancreatitis and to investigate the effects of L-ornithine using an in vitro cell culture based BBB model. At the peak of the pancreatic oedema 24 h following L-ornithine treatment (3g/kg) increased BBB permeability was measured for fluorescein and albumin. By electron microscopy oedema was observed in brain endothelial cells and astroglia endfeet. Changes in glycocalyx continuity, tight junction, plasma and basal membrane structure, and damaged mitochondria were found in endothelial cells from both brain and pancreas. As an in vitro model of the BBB primary rat brain endothelial cells, pericytes and astrocytes were co-cultured using cell culture inserts with porous membranes (Transwell, Costar). Decreased resistance and increased permeability for fluorescein and albumin were measured, and changes in endothelial cell morphology similar to those in vivo were observed in the cell culture based model. In conclusion, BBB leakage with membrane and glycocalyx damage was described during ornithine-induced pancreatitis in rats that may contribute to the development of pancreatic encephalopathy.

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Adherens junctions and extracellular matrix remodeling form a size selective barrier following focal astrocyte loss and post-translational occludin modification.

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Blood-brain barrier (BBB) dysfunction is a feature of multiple sclerosis, ischemic stroke and other neurological disorders. The mechanisms and interactions between astrocytes, extracellular matrix and vascular endothelial cells in regulating BBB integrity are poorly understood. We have previously shown that a transitory loss of astrocytes in the rat inferior colliculus induced by 3-chloropropanediol, results in reversible disruption of tight junction complexes and BBB integrity. However, BBB integrity to dextran (10-70 kDa) and fibrinogen was restored in the absence of paracellular claudin-5, occludin and ZO-1. In the present study we show increased occludin phosphorylation as BBB integrity is lost 2-6 days post 3-chloropropanediol administration. Paracellular adherens junction protein (VE-cadherin and beta-catenin) expression was maintained in vascular endothelial cells lacking paracellular claudin-5 expression. The extracellular matrix, visualized by laminin and fibronectin, showed extensive remodeling and deposition. Profiles around vascular endothelial cells became thickened and irregular with deposition within the parenchyma of the inferior colliculus. By 8-28 days there was reduced occludin phosphorylation, tight junction proteins were restored to paracellular domains and extracellular matrix profiles resembled control tissue as astrocytes repopulated the lesioned area. Extracellular matrix receptors may play a key role in regulating signal transduction between these cell–extracellular matrix and cell–cell adhesion events. Integrin subunit alpha-V showed transitory increased expression (38 kDa band) at 2-3 days, while integrin beta-3 (74 kDa band) showed increased expression at day 1, followed by decreased expression over 2-3 days before returning to control levels by 6 days. This study supports the hypothesis that a combination of adherens junctional proteins and a remodeled basement membrane, mediated in part through integrin receptors, provide a temporary size-selective barrier limiting extravasation of macromolecules and potentially neurotoxic substances into the parenchyma until tight junction proteins are restored to paracellular domains.

Applications of an improved porcine brain endothelial cell (PBEC) model of the BBB

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For many studies of blood-brain barrier function, *in vitro* models offer advantageous preparations for examination of mechanistic detail. The initial models were derived from primary cultured brain endothelial cells, and now a number of useful immortalized cell lines have also become available. However, for the most complete BBB phenotype, primary cultured models still have several advantages, chiefly the ability to make tight cell monolayers with well-organized tight junctions, good preservation of apical-basal polarity for transporters and receptors, and expression of the several mechanisms by which solutes can move or be transported across the cells. This means that the most reliable *in vitro* models for permeability screening (e.g. of drugs, biologics) are based on primary cultures. We have continued to optimize, validate and explore the applications of PBECs, as a highly practical *in vitro* BBB model. The cells can be used as monolayers, or as co-cultures grown above other cells of the neurovascular unit; for certain applications we use endothelial–astrocyte co-cultures. We will report on methods to improve the dynamic range of the model for permeability measurements, mechanistic studies on drug permeability, studies with synthetic antibody fragments that reveal aspects of glycocalyx function, and applications to test the ability of cytotoxic agents attached to dendrimer delivery agents to by-pass brain endothelial P-gp for treatment of brain tumours.

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