Sixth European Workshop

Frankfurt, Germany
Holiday Inn Hotel

March, 9th-11th 2012

BRAINS FOR BRAIN

European Task Force on Brain and Neurodegenerative Lysosomal Storage Diseases

Fondazione BRAINS FOR BRAIN- Onlus Via Giustiniani 3 c/o Dipartimento di Pediatria Salus Pueri - 35128 Padova - Italy
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Lysosomal Storage Disorders (LSDs) are inherited metabolic disorders due to the deficit of lysosomal enzymes causing accumulation of mucopolysaccharides which is responsible for cell apoptosis with time.
Lysosomal enzymes being ubiquitous molecules, their deficiency has important effects in all organs, in particular the central nervous system (CNS), liver, spleen, heart and bones.
With the advent of recombinant DNA technology, the identification and cloning of all the known lysosomal enzymes has been recently achieved, and therefore, expression and purification of recombinant proteins is now possible and enzyme replacement therapy (ERT) is now available for a growing number of storage disorders.

However, although ERT has proven to be valuable to possibly change the clinical history of the disease it has been evident that the recombinant proteins do not have any effect on the CNS, as they are unable to cross the blood brain barrier.
Furthermore, the mechanisms and etiology of CNS pathology in LSDs are still poorly understood.

We still do not know whether storage and accumulation of mucopolysaccharide is really the “primum movens” of the metabolic disaster or whether other processes might be more important (inflammation, alteration of ion channel activity, lack of chaperone molecules etc.). The understanding of these basic aspects might be extremely valuable to unravel why most of the LSDs have an attenuated and a severe form without and with CNS involvement, respectively, depending on whether there is a total enzymatic deficiency or not.

THE BRAINS FOR BRAIN TASK FORCE

The task force takes advantage from the expertise of the most distinguished european scientists, leaders in basic and applied neurotechnology and neurology grouped together to create a coordinate effort toward the comprehension of the pathophysiological processes of the neurological disorders, the implementation of knowledge on the blood brain barrier and the development of new molecular and or biochemical strategies to overcome the blood brain barrier and treat neurological disorders.
The B4B nickname of the group has been created to acknowledge the effort of the 4 initial industrial sponsors (ACTELION, BIOMARIN, GENZYME and SHIRE Human Genetic Therapies) without the support of which this brainstorming pannel could not have been created.

Brains For Brain (B4B) was formally founded in March 2007 as a research group formed by international specialists and leaders on clinical and basic research in the field of neuro-pediatrics and neuroscience.
The group has attracted interest from major biotech companies working on the development of new therapeutical strategies for lysosomal diseases, and furthermore has a strong interaction with international family associations, involved in taking care of the needs of lysosomal patients, and has stimulated collaborations toward coordinate actions to disseminate knowledge about the diseases.

B4B has also collaborated with International Scientific Associations, such as the European Study Group for Lysosomal Diseases (ESGLD) and the International Blood Brain Barriers Society (IBBS) and it is a member of the European Brain Council.

THE BRAINS FOR BRAIN FOUNDATION

The BRAINS FOR BRAIN FOUNDATION is a no-profit international organization addressed to disabled children who are affected (or healthy carriers) by rare neurological diseases.
The purposes of the FOUNDATION are:

- scientific research;
- dissemination of knowledge;
- social and socio-medical assistance;
- health assistance.

In the field of Neurodegenerative Lysosomal Disorders the aims of the *FOUNDATION* are:

- to support medical and scientific research with regard to paediatric rare neurodegenerative diseases (with particular regard to Lysosomal Storage Disorders and genetic pathologies);
- to increase public awareness and interest on such diseases;
- to organize and promote national and international research activities;
- to coordinate and promote preclinical and clinical trials;
- to organize conferences and workshops on the abovementioned topics;
- to share cultural and scientific backgrounds with different stakeholders to implement knowledge on Neurodegenerative Disorders;
- to raise funds to support research;
- to fund fellowships or prizes;
- to campaign to increase public and stakeholders awareness to Neurodegenerative Disorders and for public fund raising.

THE EUROPEAN PARLIAMENT MEETING

On December 2nd 2010 the Brains For Brain Foundation has organised the meeting: RARE NEUROLOGICAL DISEASES OF CHILDHOOD: WE TREAT THE CHILD TO TREAT THE ADULT at the European Parliament in Bruxelles.

The main aim of the meeting is to acknowledge the growing interest of the European Union Commission in both rare and neurological disorders. By holding this meeting, B4B wished to demonstrate the unity of intent of family associations, the biotechnology and pharmaceutical industries and the scientific community in stimulating interest in rare neurological diseases. It is our belief that lessons learned from in-depth research conducted into these rare genetic neurological disorders of childhood will inform treatment of more common neurological disorders.

The Brains for Brain Foundation, together with the European Brain Council and the Lysosomal Storage Disease Patient Collaborative, has organized this meeting to give relevant stakeholders the opportunity to share views on current challenges, as well as to formulate new research strategies to improve therapy and also quality of life for patients and families affected by rare neurological disorders.

AIMS OF THE WORKSHOP

The aims of the Sixth Meeting of the Brains For Brain Foundation are:

- to discuss research achievements in the field of neurodegenerative disorders and Blood Brain Barrier at clinical and basic science level;
- to discuss new recent advances on natural history and pathophysiology of LSDs with particular attention to the important role of an early intervention in preventing the morbidity and mortality associated with each of the disorders;
- to discuss factors which control the entry into the brain of medicines and other therapeutic agents which may be helpful in treating central nervous diseases;
- to discuss how B4B might collaborate with the European Union to stimulate interest in the research on LSDs and Blood Brain Barrier. For this reasons representatives from EU Commission will be invited;
- to discuss collaborations with international family associations and corporations to increase knowledge about storage diseases and research projects;
- to discuss the role of the industries in driving innovation for new therapeutical approaches for true unmet needs.

Organization
Maurizio Scarpa (IT), **David Begley** (UK), Coordinators

Scientific Officer
Cinzia Maria Bellettato (IT)

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NOTES

March 9th 2012

14.00-14.10
WELCOME AND OPENING

14.15-15.00
OPENING PLENARY LECTURE
R.O. BRADY, US
Examination of new strategies for the treatment of metabolic disorders of the brain

15.00-16.45
BASIC ASPECTS AND BBB
Chair Discussants:
JM. HEARD, FR - M. VANIER, FR

15.00-15.25
J. FAWCETT, UK
Targeting the extracellular matrix to repair the damaged nervous system
Discussion.

15.35-16.00
J. EK, SE
Inflammation and blood-brain barrier in perinatal brain injury
Discussion.

16.10-16.35
D. VIRGINTINO, IT
CXCL12/CXRC4
Pair regulates human brain vascularization
Discussion.

Coffee

17.00-17.25
M. RUONALA, DE
Spatial proteomics identify a novel drug target in JNCL
Discussion.

17.35-18.00
J. BRUYÈRE, FR
Extracellular heparan sulfate oligosaccharides affect integrin signaling and cell polarization
Discussion.

18.10-18.35
F. PLATT, UK
Clinical and drug discovery implications of defective drug metabolism in NPC disease
Discussion.

18.45-19.10
M. HOROWITZ, IL
ERAD of mutant glucocerebrosidase, Gaucher disease and Parkinson disease
Discussion.

19.30 DINNER

March 10th 2012

9.00-13.30
PATHOPHYSIOLOGY AND LSDs
Chair Discussants:
T. FUTERMAN, IL - I. BLASIG, DE

9.00-9.25
R. HASELOFF, DE
Biomarker identification in the cerebrospinal fluid: “challenges and pitfalls"
Discussion.

9.35-10.00
S. BATZIOS, GR
Metalloproteases, hyaluronic acid and other extracellular proteins as biomarkers in MPSs, but also in other lysosomal diseases
Discussion.

10.10-10.35
C. STEINHÄUSER, DE
Molecular and cellular investigation of neuron-astroglia interactions: Understanding brain function and dysfunction
Discussion.

Coffee

11.00-12.45
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 1
Chair Discussants:
H. MICHELAKAKIS, GR - C. CAILLAUD, FR

11.00-11.25
G. BALDO, BR
Proteomic alterations in the mucopolysaccharidosis type I mouse hippocampus and the effect of treatments with enzyme replacement and retroviral gene therapy started at birth or at adult age
Discussion.

11.35-12.00
A. KLEIN, IL
Pathology of neuronal forms of Gaucher disease
Discussion.

12.10-12.35
A. ZIMRAN, IL
Risk of Parkinson disease in type I Gaucher disease: a referral clinic's experience
Discussion.

13.00 LUNCH

14.00-16.20
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 2
Chair Discussants:
J. KREUTER, DE - V. GIESELMANN, DE

14.00-14.25
H. GALLA, DE
Use of nanoparticles to overcome the blood brain barrier
Discussion.

14.35-15.00
J. ABBOTT, UK
A new in vitro model of the blood-brain barrier: porcine brain endothelial cells
Discussion.

15.10-15.35
S. MURO, US
ICAM targeting of nanocarriers to the CNS
Discussion.

15.45-16.10
K. DAWSON, IE
Targeting nanoparticles to the CNS: The role of the protein corona
Discussion.

Coffee

16.45-19.30
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 2
Chair Discussants:
F. WIJBURG, NL - G. ANDRIA, IT

16.45-17.10
S. THOMAS, UK
Treating Trypanosomal infection of the CNS with small molecule therapy
Discussion.

17.20-17.45
M. BÄHR, DE
New Gene therapy approaches for treatment of Parkinson's disease
Discussion.

17.55-18.20
G.C. PARENTI, IT
Synergy between pharmacological chaperones and ERT in lysosomal diseases: the model of Fabry and Pompe Disease
Discussion.

18.30-19.15
PLENARY LECTURE
E. KAKKIS, US
The use of surrogate markers in the development of rare disease treatments: challenges and solutions essential in brain-directed treatments.
Discussion.

20.30 DINNER

March 11th 2012

08.30-11.00
INNOVATIVE THERAPIES AND EUROPEAN ACTIONS
Chair Discussants:
D. BEGLEY, UK - M. SCARPA, IT

8.30-8.55
A. BOECKENHOFF, DE
Enzyme replacement therapy of Metachromatic Leukodystrophy: Arylsulfatase A-fusion proteins for improved brain delivery of enzyme activity
Discussion.

9.05-9.30
E. LLOYD-EVANS, UK
Concentrations of miglustat in the brain versus the periphery and a novel mechanism for how miglustat provides the therapeutic benefit in patients
Discussion.

9.40-10.05
C. O'NEIL, US
Intracerebroventricular (ICV) Recombinant Human Tripeptidyl Peptidase-1 (rhTPP1) enzyme replacement attenuates disease progression in a canine model of Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL)
BioMarin Pharmaceutical
Discussion.

10.15-10.30
F. STEHR, DE
The Batten Disease International Alliance

10.30-10.55
EUROPEAN BRAIN COUNCIL:
Cost of neurological diseases in the EU (TBC)
Discussion.

Coffee

11.30-13.30
B4B AND BIOTECH COLLABORATIONS
Chair Discussants:
S. GELPERINA, RU - M. HORWITZ , IL

11.30-11.55
J.L. POWELL, US
IT-lumbar delivery of investigational rhASA in preclinical models: a journey from L4 to the deep white matter
Shire Human Genetic Therapies
Discussion.

12.05-12.30
R. GABATHULER, CA
Development of Transcend (Melanotransferrin-MTf, p97) A new vector for delivery of biologics across the BBB for the treatment of brain diseases
biOasis Technologies
Discussion.

12.40-13.05
P. GAILLARD, NL
Enhanced Brain Delivery of liposomal Methylprednisolone is beneficial for the treatment of neuroinflammation
toBBB technologies
Discussion.

LUNCH AND FAREWELL
to the next 2013 meeting

NOTES

EXAMINATION OF NEW STRATEGIES FOR THE TREATMENT OF METABOLIC DISORDERS OF THE BRAIN

ROSCOE O. BRADY, M.D.
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA.

Since the demonstration of the extraordinary benefit of enzyme replacement therapy (ERT)on the systemic manifestations of Gaucher diseasein 1991, investigators have devoted substantialeffort to improve central nervous system pathology in patients with metabolic storage disorders involving the brain. Glucocerebrosidase consists of 497 amino acids to whichfour short oligosaccharide side chains are linked is too large to cross the blood-brain barrier (BBB). Hence, ERT for approximately 5% of patients with Gaucher disease that have brain involvement was out of the question at that time.Since then several innovative strategies have been reported to overcome this impediment. Pardridge and coworkers showed that fusing a monoclonal antibody to the human insulin receptor to anenzyme improves its delivery to the brain. Sly and associates reported that inactivation of carbohydrate-dependent receptor-mediated uptake of glucuronidase treated with sodium meta-periodate followed by reduction with sodium borohydride resulted in more efficient clearing of mucopolysaccharides in the brain of glucuronidase-deficient mice than animals treated with unmodified glucuronidase. Another innovation that may prove useful is the attachment of the protein transduction domain of HIV-1 Tat protein to an enzyme. Recent investigations have provided additional insight concerning the pathogenesis of enzyme deficiency disorders. For many years it was presumed that alterations of the amino acid sequence of glucocerebrosidase such as the change of leucine to proline at amino acid position 444, the most common mutation resulting in CNS pathology, reduced the catalytic activity of the enzyme. My colleagues and I recently demonstrated that the decrease of glucocerebrosidaseactivity was the result of a quantitative loss of the amount of this enzyme. Significant increases of its activity were obtained with small molecule inhibitors of histone deacetylase that cross the BBB. The effect of such materials on neuronopathic Gaucher disease and other CNS metabolic disorders is under investigation.

TARGETING THE EXTRACELLULAR MATRIX TO REPAIR THE DAMAGED NERVOUS SYSTEM

JAMES W. FAWCETT
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Repair of damage to the nervous system requires axon regeneration, plasticity and cell replacement. The extracellular matrix plays a central role in restricting these processes, mainly through the action of chondroitin sulphate proteoglycans (CSPGs). An important agent in removing this restriction has been the enzyme chondroitinase, which removes glycosaminoglycan (GAG) chains from CSPGs. CSPGs are upregulated in glial scar tissue around injuries to restrict axon regeneration, which can be enhanced with chondroitinase. An unexpected finding has been the role of CPSGs in the restriction of plasticity. This effect is mediated by cartilage-like structures surrounding neurons, known as perineuronal nets (PNNs). Plasticity, one of the main mechanisms for recovery after nervous system injury, is greatly reduced after the critical periods of childhood. This reduction depends on deposition of PNNs around some classes of neurons throughout the CNS. These contain inhibitory CSPGs, hyaluronan, link protein and tenascin R, partly produced by the neurones themselves and partly by surrounding glial cells. All neurones with PNNs express both a hyaluronan synthase enzyme and a link protein, and these are the key components that trigger the formation of the structures. Link protein knockout animals lack normal PNNs on their dendrites. Animals lacking PNNs, or after PNN digestion by chondroitinase, possess enhanced levels of plasticity into adulthood. After CNS damage they show greatly increased recovery, particularly when the enhanced plasticity is combined with appropriate rehabilitation. Inhibition by CSPGs is due to their sulfated GAGs, which bind molecules with effect on synapses such as Semaphorin3.

INFLAMMATION AND BLOOD-BRAIN BARRIER IN PERINATAL BRAIN INJURY

JOAKIM EK, BARBARA D'ANGELO, ANA A. BABURAMANI, HENRIK HAGBERG, CARINA MALLARD
Department of Neuroscience and Physiology, University of Gothenburg, Sweden

The brain is protected by an array of barrier mechanisms that is normally referred to as the blood-brain barrier (BBB). We now have a substantial amount of evidence that many of these barrier mechanisms already start functioning in the embryo soon after the blood vessels grow into neural tissue. These barriers are probably important for establishing a favourable environment for brain growth and, hence, disturbances to barrier function during this time are likely to be detrimental to brain maturity. Previous studies have indicated disturbances to the BBB do occur following inflammation and hypoxia and this may contribute to the pathogenesis of brain injury, however, most studies have focused on the adult. We have assessed BBB function after perinatal brain injury, modelling clinically what occurs after a hypoxic-ischemic event (asphyxiation) around birth. The in vivo blood-brain barrier permeability was quantitated after an hypoxic-ischemic (HI) insult in 9 day old mice. This showed that there was a rather rapid opening of the blood-brain barrier, that peaked at 6 hours after the insult and then progressively resolved over time. Importantly, brain regions with the most significant pathology also have the greatest alterations in permeability. In addition, areas with lesions were almost identical to regions with BBB breakdown to plasma proteins further correlating pathology with abnormal BBB function. This showed that both the pattern of expression and the level of some barrier proteins were significantly changed after HI. This study defines the temporal window of compromised blood-brain barrier function after HI, strongly suggest that changes to blood-brain barrier function contributes to the pathophysiology of perinatal brain injury.

CXCL12/CXCR4 PAIR REGULATES HUMAN BRAIN VASCULARIZATION

DANIELA VIRGINTINO
Department of Basic Medical Science, Human Anatomy and Histology Unit
Bari University Medical School, Bari, Italy

The blood-brain barrier (BBB) is a working system of mutual cellular and non-cellular interactions that are essential to brain function and have a major impact during brain development. In human foetal cerebral cortex, vessel growth and BBB differentiation represent parallel events reflected by the endothelial barrier phenotype and by signs of vascular and perivascular cell activation. The stem vessels, which penetrate the developing cortex by sprouting from the perineural plexus, grow radially deep into the tissue to the periventricular brain layers. In these radial vessels branching angiogenesis prevails in the subcortical layers, while in the cortex the vessels seem to fully differentiate before starting with a branching process at the time of cortex lamination. The typical radial pattern of cortex microvessels is mirrored by radial fibres of astroglia cells which serve as cables for migrating neurons and are seen to form tight contacts with the microvessel wall. In view of this very striking tendency to differentiate BBB devices shown by these growing cortex microvessels, we investigated the molecules involved in this double process, which follows the same direction but at opposite poles, and started to analyse the expression of growth factors and their receptors within the radial vessels/radial fibres couple. Among the obtained results, some findings seem to us of particular interest i.e. the disclosed differential expression of chemokine CXCL12, a potent migration and differentiation factor, by a subset of vessel-contacting radial glia cells within the cortex, and the presence of the CXCL12/CXCR4 pair in areas of vessel branching in vessel-contacting, transitional, giant forms of astrocytes. These preliminary results suggest a role for glio-vascular interactions during cerebral cortex vascularization by way of the CXCL12/CXCR4 signalling axis, which could coordinate microvessel elongation and branching with BBB differentiation and neuroblast migration.

SPATIAL PROTEOMICS IDENTIFY A NOVEL DRUG TARGET IN JNCL

ANNA I. KROKFORS[1], GERO P. HOOFF[2], PAVLINA WOLF[3], JULIANA MARCELA RAMOS MORENO[1], ANTON PETCHERSKI[1], REYK HILLERT[4], WALTER SCHUBERT[4], SUSAN L. COTMAN[3], GUNTER P. ECKERT[2], MIKA O. RUONALA[1]
[1] NeuroToponomics Group, Center for Membrane Proteomics, and [2]Department of Pharmacology, University of Frankfurt am Main, Frankfurt am Main, Germany, [3] Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA,USA, [4]MPRR Group, Medical Faculty, University of Magdeburg, Magdeburg, Germany

The fact that the pathogenesis of Juvenile Neuronal Ceroid Lipofuscinosisremain unresolved largely accounts for the enigmatic CLN3 protein. Here, we approached the JNCL disease mechanisms from the top-down perspective of spatial proteomicsusing Multi-Epitope-Ligand-Cartography (MELC). The direct visualization of dozens of JNCL associated cellular components in situ in the hippocampus of an adult genetically precise JNCL mouse model(CD1-CLN3**d**ex7/8) led to the identification of a novel, drugable metabolic phenotype for JNCL.

results

The architecture of the protein network formed by over 30 cellular components showed remarkable rearrangement in situin the hippocampus of adult male JNCL mice in comparison to control animals. Careful inspection of the MELC data suggested that the deviations could originate from defect on a metabolic pathway. Acknowledging the slow progression of JNCL we hypothesized that a drug-mediated temporal modulation of the activity of this pathway could have an influence on the disease progression in the CNS. Indeed, in vitro in CLN3 CLN3dex7/8cerebellar granular cells a short-term inhibition of the pathway efficientlyre-established the well-known lysosomal phenotype of JNCL towards wild-type cells as well as induced a clearance of proteolipid accumulates. In vivo treatment introduced a halt in the progression of the disease in vivo in adult male CD1-CLN3dex7/8 mice as judged by behavioral studies. Subsequent analyses showed that the rescue mechanism triggered by the treatment involved at least enhanced autophagy, reduced mitophagy, and enhanced endo-lysosomal function.

conclusions

Our work here demonstrates the power of the genetically precise JNCL mouse model and our top-down proteomics strategy leading to a potential therapy form. We propose that the pathogenesis of JCNL and possibly other NCL forms can be therapeutically influenced with correctly targeted drugs.

EXTRACELLULAR HEPARAN SULFATE OLIGOSACCHARIDES AFFECT INTEGRIN SIGNALING AND CELL POLARIZATION

JULIE BRUYÈRE[1], ELISE ROY[1], STÉPHANIE BIGOU[1], PATRICIA FLAMANT[1], JEAN-MICHEL HEARD[1] AND SANDRINE VITRY[1]
[1] Institut Pasteur, Department of Neuroscience, Rétrovirus et Transfert Génétique, INSERM U622, Paris, France

Mucopolysaccharidosis type IIIB (MPSIIIB, Sanfilippo syndrome B) is a lysosomal storage disease characterized by progressive mental retardation and neurodegeneration in children. This rare monogenic disease is caused by alpha-N-acetylglucosaminidase (NAGLU) deficiency, a lysosomal hydrolase necessary for heparan sulfate (HS) degradation. This deficiency leads to the accumulation of heparan sufate oligosaccharides in brain cells and extra-cellular environment. Mechanisms mediating HS oligosaccharides deleterious effects on brain cells are not well understood.
Heparan sulfate at the extra-cellular environment are essential for adequate growth factor signaling and integrin functions. Integrin clustering and colocalization with their associated signaling and cytoskeletal molecules form focal adhesions. The recruitment of integrins and the turnover of focal adhesions are essential for the regulation of cell adhesion, polarization and motility.

Here, we provide evidence that cell sensing of environment is altered in MPSIIIB cells. Beta1 integrin and focal adhesion components are over-recruited and over-activated in deficient astrocytes. HS oligosaccharide clearance, by NAGLU gene transfer, rescues a normal phenotype suggesting that HS oligosaccharides induce focal adhesion formation. Addition of purified HS oligosaccharides on normal astrocytes confirms that extracellular HS oligosaccharides can activate the recruitment of focal adhesion components. Consistently, integrin-dependant cell behavior such as cell polarization was defective in oriented migration assays performed in deficient astrocytes and deficient neural stem cells.

HS oligosaccharides affect cell sensing of extra-cellular cues with consequences on cell polarization. Accumulation of these compounds in affected newborn brain may affect cell migration and neuritogenesis and have deleterious consequences on central nervous system development.

CLINICAL AND DRUG DISCOVERY IMPLICATIONS OF DEFECTIVE DRUG METABOLISM IN NPC DISEASE

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[2] Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institutes of Health, Department of Health and Human Services, Bethesda, MD
[3] Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD
[4] University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA.

Niemann-Pick type C (NPC) disease is a neurodegenerative lysosomal storage disease caused by mutation in either the NPC1 or NPC2 genes. It is characterized by storage of multiple lipids in the late endocytic compartment and reduced levels of acidic store calcium, leading to a block in late endosome:lysosome fusion. The underlying molecular mechanisms that lead to pathogenesis are not fully understood. Authentic mouse and cat models of NPC1 disease are available.
When evaluating small molecule therapeutics in the NPC1 mouse we observed a consistent pattern of toxicity associated with administration drugs that are metabolized via the cytochrome P450 system, but not with drugs such as miglustat that is excreted intact via the kidney. These observations suggested a potential drug metabolism defect in the NPC disease. We therefore investigated the P450 system and found that NPC1 disease is associated with a significant reduction in the expression and activity of cytochrome P450 reductase in the NPC1 cat and mouse models. The activities of multiple cytochrome P450 catalyzed dealkylation reactions were reduced in both NPC1 mouse and cat models and in liver samples from NPC patients. In vivo drug metabolism studies using a prototypic P450 metabolised drug, Midazolam, confirmed that the kinetics of drug clearance was impaired in NPC1 mice. These finding have implications for drug testing in animal models and potentially the clinical management of NPC patients.

ERAD OF MUTANT GLUCOCEREBROSIDASE, GAUCHER DISEASE AND PARKINSON DISEASE

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There is a growing number of protein deficiencies that result from mutations causing misfolded protein conformation. Such are mutant lysosomal enzymes, whose inability to correctly fold in the ER leads to their retrotranslocation to the cytoplasm, their ubiquitination and proteasomal degradation in a process known as the ER associated degradation (ERAD). ERAD is also associated with unfolded protein response (UPR).
Gaucher disease (GD), a sphingolipid storage disorder, characterized by impaired activity of the lysosomal enzyme glucocerebrosidase (GCase), results from mutations in the glucocerebrosidase encoding gene (GBA). We have shown that mutant glucocerebrosidase variants undergo ERAD, which is accompanied by UPR.
An association has been demonstrated between GD and Parkinson disease (PD) by the concurrence of GD and parkinsonism in patients and the identification of GCase mutations in probands with sporadic PD. One of the proteins involved in PD is the E3 ubiquitin ligase parkin.
We tested the possibility that the concurrence of GD and PD reflects the fact that parkin is an E3 ubiquitin ligase of misfolded mutant GCase variants. We could show that mutant GCase variants undergo ubiquitination and proteasomal degradation, mediated by parkin. Overexpression of a known parkin substrate leads to accumulation of mutant GCase, confirming that parkin mediates degradation of mutant GCase. More so, knockdown of parkin is associated with stabilization of mutant GCase. Dopaminergic cells expressing mutant GCase are more vulnerable to apoptosis.
We suggest that involvement of parkin in degradation of mutant GCase leads to death of dopaminergic cells and explains the concurrence of GD and PD.

BIOMARKER IDENTIFICATION IN THE CEREBROSPINAL FLUID: CHALLENGES AND PITFALLS

R.F. HASELOFF, I.E. BLASIG, E. KRAUSE, M. SCHÜMANN, J. AUSSEIL,* AND J.M. HEARD**
*Leibniz-Institut für Molekulare Pharmakologie, Berlin-Buch, Germany; *Laboratoire de Biochimie, INSERM U1088, Centre Hospitalier Universitaire d'Amiens, France; **Pasteur Institute, Paris, France*

Specific biomarkers are highly valuable tools for early diagnosis, estimation of disease progress and treatment efficacy in patients suffering from neurodegenerative disorders. The cerebrospinal fluid (CSF) directly contacts the brain and, hence, represents a reliable indicator for the functional, pathological, and pharmacological status of the brain. The CSF contains a defined number of proteins which makes it well suitable for proteomic analyses. The detection of the potentially more predictive low abundant proteins is hindered by high abundant proteins, such as albumins or globulins, representing more than 75% of the protein content in the CSF. Consequently, a major problem of CSF proteomics is depletion of these dominating proteins. We report about our methodical efforts to decrease the concentration of the major proteins using different procedures applied for CSF of different species. Furthermore, we analysed the CSF of dog models for the neurodegenerative lysosomal storage diseases mucopolysacchrosidosis III B (MPSIIIB, Sanfilippo syndrome type B) and I (MPSI, Hurler syndrome) by mass spectrometry. Alterations in the protein profile of the CSF, caused by MPS compared to the healthy control were further verified by alternative methods, such as immunoblotting. The data will be discussed in the context of the literature, and conclusions will be drawn for the further validation of biomarker candidates.

METALLOPROTEASES, HYALURONIC ACID AND OTHER EXTRACELLURAR PROTEINS AS BIOMARKERS IN MPSS, BUT ALSO IN OTHER LYSOSOMAL DISEASES

SPYROS BATZIOS, MD, PHD
Medical School, Aristotle University of Thessaloniki

The extracellular matrix (ECM) represents a physical scaffold for cell and tissue organization, while also is a microenvironment providing molecular and spatial information that influences multiple cell processes. Through its function, it regulates growth factors, chemokine and cytokine availability, contributes to tissue homeostasis maintenance, influences cell proliferation, differentiation and survival. ECM is composed of various molecules, presenting dynamic interactions with each other and with different types of cells. Among those molecules, matrix mettalloproteinases and their tissue inhibitors, hyaluronic acid and various proteoglycans represent ECM components which have been implicated in various cell processes, both normal and pathological, usually related to cell apoptosis and inflammation. The mucopolysaccharidoses (MPS) represent a heterogeneous group of hereditary disorders characterized by the accumulation of glycosaminoglycans (GAGs) within the lysosomes. Although crucial steps have been made towards understanding the full aetiopathogenetic repertoire of MPSs, the exact mechanisms by which deficiencies of lysosomal hydrolases ultimately lead to disease manifestations are not clear. Recent findings indicate that the primary accumulation of GAGs within the lysosomes may trigger a cascade of events which influence various biochemical and physiological processes of the cell. In addition, there is emerging evidence for the involvement of inflammation in the pathophysiology of MPS. Accordingly, several mediators of the inflammatory response have been tested as possible molecular biomarkers for these disorders. The goal of this presentation is to review recent findings concerning the expression of ECM components in patients with MPSs and other lysosomal storage disorders. The possible involvement of ECM molecules in the aetiopathogenesis of this group of diseases will provide new insights into the molecular mechanisms of these ailments, unravel potential biological markers for the diagnosis, follow-up and response to therapy and finally orientate research towards the use of those molecules as pharmaceutical targets.

SixthEuropeanWorkshop

MOLECULAR AND CELLULAR INVESTIGATION OF NEURON-ASTROGLIA INTERACTIONS: UNDERSTANDING BRAIN FUNCTION AND DYSFUNCTION

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Glial cells are now recognized as active communication partners in the CNS, and this new perspective has rekindled the question of their role in pathology. We observe unusual immunohistochemical and functional phenotypes of glial cells surviving in the sclerotic hippocampus (HS) of patients with temporal lobe epilepsy (TLE), including a complete loss of gap junction coupling. It is, however, unclear whether these changes reflect the cause, effect or adaptive response in the progression of epilepsy. To gain insight into the temporal relationship between seizure development and astroglial uncoupling we have established a mouse model of epilepsy (unilateral intracortical kainate injection) which reflects many key aspects of human TLE. Changes in interastrocytic coupling were assessed by tracer diffusion studies in acute slices from mice at different time points post status epilepticus. These studies revealed a pronounced reduction of coupling already during the latent period (i.e. before onset of spontaneous seizures) and a complete loss of coupling during the early chronic phase, providing strong evidence that this dysfunction is a crucial factor in epileptogenesis. To evaluate the mechanism(s) underlying the loss of astrocytic coupling in HS we examined Cx43 mRNA and protein expression in the sclerotic hippocampus of transgenic reporter mice (Cx43ki-ECFP) with real-time RT-PCR and Western blot analysis. The results from these studies indicate that posttranslational modifications rather than downregulation of Cx43 expression account for gap junction inhibition in epilepsy. Using the Cre-lox fate mapping strategy for permanent labelling of astrocytes, we gained further support for the notion that astrocytes in HS do not transdifferentiate into another cell type but rather aquire another functional phenotype. Together, these data challenge the common view of epileptogenesis according to which changes in neurons are considered the prime cause of this condition.

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PROTEOMIC ALTERATIONS IN MUCOPOLYSACCHARIDOSIS TYPE I MOUSE HIPPOCAMPUS AND THE EFFECT OF TREATMENTS WITH ENZYME REPLACEMENT AND RETROVIRAL GENE THERAPY STARTED AT BIRTH OR AT ADULT AGE

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Proteomic analysis is a powerful tool to study abnormalities responsible for pathogenesis of several diseases and therefore in the present work we aimed to identify proteins possibly involved in the cognitive loss in MPS I mice, as well as the effects of enzyme replacement (ERT) and retroviral gene therapy (GT) started at neonatal period or at an adult age.
We dissected hippocampus from 8-month old normal and MPS I mice, and submitted these samples to shotgun proteomic analysis. We identified 296 proteins, of which 32 were differentially expressed ($p<0.05$). Among upregulated proteins, we found proteins involved in sphingolipid metabolism (Prosaposin, 5.6-fold), lysosomal enzymes (cathepsin D, 7.5-fold), neuroinflammation (glial fibrillary acid protein, 4.5-fold), signal transduction (mitogen-activated protein kinase-1, 2.9-fold), glutamate metabolism (glutamine synthethase, 1.25-fold) and others. Among downregulated proteins, genes shown to be involved in synaptic maturation like microtubule-associated proteins 1B (0.2-fold) and 1A (0.4-fold), synaptophysin (0.6-fold) were identified. We confirmed the elevation in cathepsins using fluorogenic substrates, and since they can mediate cell death, we performed TUNEL staining, as well as an enzyme assay for caspases. Surprisingly, we didn't find convincing signs of significant cell death in MPS I neurons, suggesting that a neuronal dysfunction rather than neuron death is responsible for the cognitive impairment.
Based on those findings, we treated MPS I mice with ERT (1.2mg/kg every 2 weeks) and GT from birth or from 2 months, which is the time when first behavioral abnormalities were described. We verified that a small fraction of the enzyme passes the blood-brain-barrier using both approaches, and that all treated groups showed improvement in behavior tests.
Taken together our results suggest that neuron dysfunction is responsible for cognitive loss in MPS I mice. These results might have important implications when translating into patients, since they might have some benefit from treatment, even when started later in life.

PATHOLOGY OF NEURONAL FORMS OF GAUCHER DISEASE

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Gaucher disease (GD), the most predominant Lysosomal Storage Disorder (LSD), is caused by the defective activity of lysosomal acid-**b**-glucosidase (glucocerebrosidase, GCase), resulting in accumulation of the glycosphingolipid, glucosylceramide (GlcCer). The disease can be divided into 3 major subtypes, of which types 2 and 3 result in severe neurological disorders in patients. Despite years of study of the genetic and molecular bases of GD, little is known about the events that lead from intra-lysosomal accumulation to the distinctive cell dysfunction and pathology. We have systematically analyzed the neuropathology of GD animal models and have identified brain areas that are susceptible and/or resistant to cell death and we are currently trying to understand what regulates these processes. We are evaluating potential correlations between lipid accumulation and neuronal death as well as the gene expression patterns of the affected and non-affected brain areas in order to identify novel pathways that might be involved in GD cell death; are these pathways the same in all affected areas? Finally, we are also investigating why GD patients present different subtypes of GD. To answer these questions, we are using the available mouse models of GD and developing new genetic and chemical animal models that ultimately will allow us to design and test new therapies for this devastating disease.

This research is funded by Children's Gaucher Research Fund.

RISK OF PARKINSON DISEASE IN TYPE 1 GAUCHER DISEASE: A REFERRAL CLINIC'S EXPERIENCE

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Background: While type 1 Gaucher disease (GD) patients have an increased risk for Parkinson disease (PD), it is unknown whether certain GD phenotypes are associated with an increased PD risk.

Aim: To present the clinical characteristics of adults affected by both GD and PD from a large Israeli GD clinic.

Methods: Medical files of patients >18 years between 1990-2010 were reviewed for PD. Available patients with suspected parkinsonism underwent an additional neurological examination by a movement disorders specialist (MDS). Demographic and GD characteristics were compared between GD patients with and without PD using t-test and Fisher exact tests.

Results: 510 type 1 GD adults (233 males; 45.7%) had records reviewed. 12 patients with suspected parkinsonism were identified; 11 were confirmed by an MDS, using UK brain bank criteria for PD (2.2%). Among those with GD/PD, cognitive impairment was common (7/11). Two patients underwent successful deep brain stimulation (DBS). PD diagnosis was associated with male gender (81.8% versus 44.9% male, p=0.027) and older age (mean age, GD/PD=62.8, GD non-PD=47.1, p=0.004). GD phenotype and severity did not differ between the two groups, including mean Zimran Severity Score index for GD severity (7.7 versus 8.3), percent splenectomized (15.8% versus 27.3%), history of avascular necrosis (13.0% versus 27.3%) and percent ever treated with enzyme replacement (49.4% versus 45.5%).

Conclusion: While GD is associated with PD, GD severity is not associated with increased risk for PD. Further research is required to assess which GD patients are at a higher risk for PD, and why.

USE OF NANOPARTICLES TO OVERCOME THE BLOOD BRAIN BARRIER

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Nanoparticles have been widely used as carriers to transfer drugs across the blood brain barrier. Following this approach we usedpolysorbate 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. Fe3O4is 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.

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A NEW IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER: PORCINE BRAIN ENDOTHELIAL CELLS

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The neurones of the brain and spinal cord (central nervous system, CNS) require precise control of their bathing microenvironment for optimal function, and an important element in this control is the blood-brain barrier (BBB). The BBB is formed by the endothelial cells lining the brain microvessels, under the inductive influence of neighbouring cell types within the 'Neurovascular Unit' including astrocytes and pericytes. The endothelium forms the major interface between the blood and the CNS, and by a combination of low passive permeability and presence of specific transport systems and enzymes, regulates molecular and cellular traffic across the barrier layer. In vitro models of the BBB are valuable tools for investigation of normal BBB physiology, of ways in which is may be disturbed in pathology, and of strategies for optimizing drug delivery to the CNS.

Our group has developed and worked with a number of in vitro BBB models, using both primary cultures and immortalized cell lines, but recently we have focused on a primary culture model of porcine brain endothelial cells (PBECs), which has several advantages. It is straightforward to produce, and with careful preparative procedures gives good reproducibility. It generates tight cell monolayers (transendothelial electrical resistance TEER >500-1000ohm.cm2) and expresses key BBB transport systems (uptake, efflux), giving good resolution and discrimination for studies of permeation of small molecules including drugs. Co-culture with primary rat astrocytes increases TEER, apical/basal polarity in transporter expression, and functional peptide and protein transport, both by receptor-mediated and adsorptive-mediated transcytosis. Variants of the model can be used to mimic several CNS pathologies e.g. tumours, hypoxia, inflammation, giving valuable mechanistic information. This PBEC model is being used for a range of studies on drug permeation through the BBB, including delivery of peptides, proteins and artificial constructs with potential in treatments of lysosomal storage diseases.

ICAM-1 TARGETING OF NANOCARRIERS TO THE CENTRAL NERVOUS SYSTEM

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Transport across the blood-brain barrier (BBB) is a major obstacle for treatment of medical conditions affecting the central nervous system (CNS). This is the case for many lysosomal storage disorders (LSDs), where the BBB hinders the efficacy of enzyme replacement therapy and other therapeutics administered intravenously. Current promising approaches are exploring the use of drug nanocarriers to develop non-invasive means for BBB transport, while minimizing permeability safety concerns. Nanocarriers have the potential to improve drug solubility, circulation time, protection from rapid degradation, targeting to specific sites, and timed release. Targeting of nanocarriers to molecules expressed on brain endothelial cells involved in BBB transport can improve access into the CNS. We are exploring such a strategy by coupling lysosomal enzymes to polymer nanocarriers targeted to intercellular adhesion molecule 1 (ICAM-1). ICAM-1 is a transmembrane glycoprotein expressed on endothelial cells (including the BBB) and other cell types through the body (e.g., neurons). It is up-regulated by many pathologies, facilitating targeting to disease sites. Using various in vitro, cell culture and mouse models, we have demonstrated that targeting ICAM-1 with nanocarriers induces endocytic transport by a non-classical mechanism, cell adhesion molecule (CAM)-mediated endocytosis, different from clathrin- and caveolar-mediated pathways. In cellular barriers, such as those pertinent to the BBB and the gastrointestinal epithelium, ICAM-1-targeted nanocarriers are transported across cells by a transcytosis mechanism related to the CAM pathway, without disruption of the cell junctions that maintain the permeability barrier. The efficacy of this transport is high within a broad spectrum of nanocarrier formulation parameters, including composition, size, shape, valency, and loaded cargo. This strategy markedly enhances lysosomal enzyme delivery to peripheral organs and the brain, with improved internalization within cells, transport to lysosomes, and enhanced biochemical effects. Therefore, this platform holds considerable potential regarding development of LSD-aimed therapeutic interventions.

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TREATING TRYPANOSOMAL INFECTION OF THE CNS WITH SMALL MOLECULE THERAPY

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Human sleeping sickness (Human African Trypanosomiasis) is caused by a parasite. It causes death if left untreated. The type of drug offered to patients depends on the presence of the parasite in the brain. If the disease has progressed to this stage, then the drug must reach this restricted site. However, most of the existing drugs to treat this disease were developed over 40 years ago and no study had, until recently, directly investigated the ability of these drugs to reach the healthy or diseased brain. This issue is of special concern because in some patients single drugs no longer work. Consequently, two drugs are being used together with little understanding about their therapeutic effect and it has been proposed that, in some cases, this effect is a result of improved drug entry into the brain. Although 60 million people are exposed to this disease, companies have not been interested in developing new drugs due to the lack of financial reward. No new drugs will be available for at least 10 years. A clearer understanding of drug distribution to the brains of sleeping sickness patients is needed if we are to use treatments that are more effective and safe. We will present our latest studies in this area[1-5].

[**1**] *Brain Research in press* (10.1016/j.brainres.2011.11.053). [**2**] *J. Pharm. Exp. Ther.* 336(2011) 506-515. [**3**] *J. Pharm. Exp. Ther.* 329(2009) 967–977. [**4**] *J. Neurochem* 107 (2008) 1136-1146. [**5**] *Antimicrobial Agents and Chemotherapy* 51 (2007): 3136-3146.

NEW GENE THERAPY APPROACHES FOR TREATMENT OF PARKINSON'S DISEASE

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The majority of human neurodegenerative diseases develop with increasing age and show a multifaceted pathology with signs of synaptic dysfunction, oxidative stress and axonal degeneration preceding neuronal cell loss. The latter often displays features of programmed cell death. The pro-apoptotic signals that have been described so far seem to initiate breakdown of mitochondrial membrane potential, followed by the release of proapoptotic factors from the inner mitochondrial membrane and subsequent caspase activation. Thus, to develop a versatile neuroprotective therapy for several different disorders that makes the affected neurons more resistant to stress stimuli one needs to focus on the maintenance of mitochondrial integrity e.g. by overexpression of antiapoptotic members of the Bcl-2 family of proteins, neurotrophic factors or other upstream regulators of mitochondrial morphology and membrane integrity. This approach, in contrast to inhibition of downstream events after AIF or cytochrome C release, apoptosome formation and Caspase activation may prove much more effective in protecting affected cells from dysfunction and subsequent cell death. Glial cell line-derived neurotrophic factor (GDNF) is a promising candidate in the long-term treatment of Parkinson's disease, but its route of application is complicated and may explain the variable outcome in recent clinical trials.
To elucidate effective and long-lasting neuroprotective strategies, we analysed a combination of mitochondrial protection and neurotrophic support in two well-defined animal models of neurodegeneration, traumatic lesion of the optic nerve and 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. Neuroprotection by BclXL, Glial cell line-derived neurotrophic factor (GDNF) or BclXL combined GDNF co-expression were studied using modified AAV for gene transfer. We could show that BclXL expression is more important for neuronal survival in the early phase after lesions, whereas GDNF-mediated neuroprotection becomes more prominent in the advanced state of neurodegeneration.
To further develop this approach, we have successfully applied and been rewarded with a new grant in the 7th EU-Framework programme (FP7) where we have developed more sophisticated AAV and Lentivirus based Gene Therapy techniques in a consortium of the major academic centres in Europe together with a Biotech company. To that end, we have recently achieved long-lasting, cell-type specific, regulatable vector systems for CNS gene therapy with low or no immunogenicity. The presentation will include the new findings that result from this cooperation and from the scientific interactions within the research focus 'Aggregation Disorders' within the Cluster of Excellence at German Elite University Göttingen.

SYNERGY BETWEEN PHARMACOLOGICAL CHAPERONES AND ERT IN LYSOSOMAL DISEASES: THE MODELS OF FABRY AND POMPE DISEASE

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Pharmacological chaperone therapy (PCT) has been recently evaluated as a therapeutic strategy for the treatment of lysosomal storage diseases. This approach has been traditionally designed for the treatment of diseases due to protein misfolding, by using small-molecule ligands that increase stability of mutated proteins and prevent their degradation [Fan, 2008; Parenti, 2009; Valenzano et al, 2011]. Recent studies, however, have shown that chaperones are not only able to rescue misfolded defective proteins, but may also potentiate the effects of the wild-type recombinant enzymes used for enzyme replacement therapy (ERT).
In the recent years we have studied the synergy between PCT and ERT in two among the most prevalent lysosomal disorders, Pompe disease (PD) and Fabry disease (FD) [Porto et al, 2009; Porto et al, 2011].
PD is caused by the functional deficiency of alpha-glucosidase (GAA), an acid hydrolase involved in the lysosomal breakdown of glycogen. GAA deficiency results in glycogen accumulation in lysosomes and in secondary cellular damage [van der Ploeg and Reuser, 2008; Parenti and Andria, 2011]. The disease manifestations are predominantly related to the involvement of cardiac and skeletal muscles with variable association of severe cardiomyopathy, muscle hypotonia, motor impairment and respiratory failure. The clinical course is progressive and impacts severely on the health of PD patients.
Fabry disease (FD) is an X-linked inherited disease due to alpha-galactosidase A (alpha-Gal A) deficiency and characterized by lysosomal storage of globotriaosylceramide (Gb3) and related neutral glycosphingolipids. Storage of these substrates results in multisystem manifestations, including renal failure, cardiomyopathy, premature myocardial infarctions, stroke, chronic neuronopathic pain, gastrointestinal disturbances, and skin angiokeratoma.
For both diseases ERT with recombinant human GAA (rhGAA) and recombinant human alpha-Gal A (rh-alpha-Gal A), respectively, became available in the early 2000s. However, in both cases ERT efficacy may vary in different tissues and its long-term effects remain to be defined.
We have shown that, when recombinant enzymes were administered to mutant PD and FD fibroblasts in combination with the chaperone molecules N-butyl-deoxynojirimycin (NB-DNJ) and 1-deoxy-galactonojiirimycin (DGJ), respectively, the lysosomal trafficking, the maturation and intracellular activity of the enzymes increased. Improved stability of rhGAA was also observed in PD fibroblasts. In FD fibroblasts the clearance of lyso-Gb3, one of the substrates stored in FD and a potent inhibitor of GLA, was also significantly improved with the co-administration of DGJ and rhGLA.
Although for both disorders clinical translation of these studies is already in progress with phase 2 trials, the molecular mechanisms underlying the synergy between PCT and ERT remain to be fully elucidated. Important aspects of chaperones' action remain poorly characterized and information is lacking on their intracellular distribution, inhibition of resident enzymes in specific cellular compartments, and specificity. A reason of major concern on the clinical use of chaperones is the fact that in most cases these drugs are competitive inhibitors of target enzymes [Valenzano et al, 2011].

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ENZYME REPLACEMENT THERAPY OF METACHROMATIC LEUKODYSTROPHY: ASA-FUSION PROTEINS FOR IMPROVED BRAIN DELIVERY OF ENZYME ACTIVITY

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disease which is caused by a functional deficiency of the lysosomal enzyme Arylsulfatase A (ASA). The lack of ASA activity leads to the intralysosomal accumulation of the ASA substrate sulfatide, progressive demyelination of the CNS, rapidly deteriorating neurological symptoms and early death. Curative therapy is not available. Based on preclinical data in MLD mouse models, enzyme replacement therapy (ERT) has been suggested to be a treatment option. However, only a small fraction of recombinant human ASA injected into the mouse circulation reaches the brain parenchyma, such that high ASA doses are required for therapeutic benefit. Consequently, strategies to increase the low transfer rate of recombinant enzyme across the blood-brain barrier (BBB) are required to improve the efficacy of this approach.
Experiments in a porcine cell culture model of the BBB suggested that the transendothelial transport of ASA follows two independent routes, being due to mannose 6-phosphate receptor-mediated transcytosis and adsorptive-mediated transport, respectively (Matthes et al., 2011). In an attempt to increase brain delivery of ASA activity, we generated fusion proteins between human ASA and peptides which had been shown previously to mediate efficient transfer of cargo molecules across the BBB. These peptides include the protein transduction domain of the HIV TAT-protein and the receptor binding domains of apolipoprotein B (ApoB) and E (ApoE). ASA-TAT, ASA-ApoB and ASA-ApoE were expressed in Chinese hamster ovary cells. Cellular uptake, intracellular targeting, specific activity, glycosylation and binding to the mannose 6-phosphate receptor and the LDL-receptor related protein (LRP) were analysed. When tested in the BBB cell culture model, ASA-ApoE, but not ASA-ApoB and ASA-TAT, showed an increased transendothelial transfer rate. Consistent with this observation, intravenously injected ASA-ApoE accumulated to significantly higher levels in total brain of ASA knockout mice compared to wildtype ASA and in contrast to ASA-ApoB and ASA-TAT.

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INTRACEREBROVENTRICULAR (ICV) RECOMBINANT HUMAN TRIPEPTIDYL PEPTIDASE-1 (RHTPP1) ENZYME REPLACEMENT ATTENUATES DISEASE PROGRESSION IN A CANINE MODEL OF LATE INFANTILE NEURONAL CEROID LIPOFUSCINOSIS (LINCL)

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BioMarin Pharmaceutical Inc.

LINCL is caused by lack of the enzyme TPP1. LINCL patients exhibit accumulation of lysosomal storage in the CNS accompanied by neurodegeneration, loss of function, and death. TPP1-null dachshunds recapitulate many symptoms of the human disease. This study was performed to determine the pharmacology of ICV rhTPP1 in this model. An additional objective was to characterize the pharmacokinetics (PK) and distribution in the CNS. Affected animals (N=9) and wild-type controls (N=9) received ICV infusions of 4 or 16mg rhTPP1 or artificial cerebrospinal fluid (CSF) vehicle. rhTPP1 was administered as two or four hour infusions via a catheter implanted in a lateral ventricle. Animals received approximately 20 biweekly doses starting at age 2 months. Serial plasma and CSF samples were collected during and after dose administration to characterize rhTPP1 PK. Elevated CSF concentrations were observed for at least 48 hours after infusion and were 500- to 1000-fold higher than plasma levels. Neurological and clinical examinations, electretinography, measurement of pupillary light reflexes and visual evoked potentials, magnetic resonance imaging, and cognitive testing were performed throughout the study to assess disease progression. Necropsy occurred 48 hours after the final dose at approximately 11 months of age. Animals administered rhTPP1 exhibited improved clinical signs and attenuated functional decline compared to vehicle treated controls. In-life assessments at the 16mg dose level, as well as analysis of CNS tissues for rhTPP1 concentration, storage material, and markers of neuronal injury, are ongoing.

THE BATTEN DISEASE INTERNATIONAL ALLIANCE

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Batten Disease International Alliance (BDIA)

The newly formed Batten Disease International Alliance (BDIA) is an organization of family-founded organizations, public charities, non-governmental organizations and foundations dedicated to accelerating Batten disease research through the strategic placement of funds. We want to offer support and information to better the lives of those affected, to promote collaboration, and to focus and drive important research in all forms of Batten disease.
Our present members represent organizations from around the world, many of whom are directly involved in national and international activities leveraged against existing programs and resources in the non-profit sector, government and for-profit agencies. Members are sharing their personal and professional experiences to provide ongoing support, reliable information and hope through research for those affected by this condition.
Representatives of BDIA member organizations meet monthly by phone and biannually in-person to share support services, educational materials and research funding information to prevent duplication, to discuss opportunities for joint funding, and to disseminate recent findings in formats that enthuse and inform their constituents. These meetings also serve as an opportunity to identify critical research needs best led or facilitated by each organization. We believe these efforts assist families living with Batten disease and speed the development of treatments for those affected by this terrible condition.

IT-LUMBAR DELIVERY OF INVESTIGATIONAL RHASA IN PRECLINICAL MODELS: A JOURNEY FROM L4 TO THE DEEP WHITE MATTER

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Shire Human Genetic Therapies

Metachromatic leukodystrophy is a devastating disease resulting from demyelination in the central and peripheral nervous systems, leading to rapidly progressive neurological symptoms and early death in patients. In the brain, cells within the deep white matter appear to be particularly vulnerable to the loss of lysosomal enzyme Arylsulfatase A (ASA) and resultant accumulation of sulfatide. A major challenge to the treatment of MLD is the successful delivery of recombinant enzyme to the CNS, particularly across the blood brain barrier (BBB). Drugs delivered directly into the cerebrospinal fluid (CSF) bypass the BBB, and we and others have previously reported the delivery and biodistribution of recombinant human ASA (rhASA) in animal models via this route. Our recent studies have focused on assessing the pharmacokinetic (PK) and pharmacodynamic (PD) parameters for rhASA in preclinical models following intrathecal-lumbar (IT-L) delivery. PK studies in cynomolgus monkeys combining PET imaging and terminal sampling suggest that distribution of rhASA throughout the spinal cord and brain occurs rapidly, with maximum levels in deep white matter 8-24 hours after dosing, and declining thereafter. As shown by immunohistochemical staining, higher levels of rhASA were found associated with white matter compared to gray matter in the cerebrum, cerebellum and spinal cord. In the PD studies, assessment of brain and spinal tissue from immunotolerant ASA knockout mice receiving four weekly IT-L doses of rhASA revealed a significant reduction in sulfatide and a decrease in LAMP-1 staining in cerebral white matter and spinal cord. Taken together these data support IT-L delivery of rhASA via the CSF for distribution to white matter of the brain and spinal cord in preclinical animal models. The specific mechanisms by which enzyme traverses the length of the spine and is distributed to deep regions of the brain are currently unknown and warrant further investigation.

DEVELOPMENT OF TRANSCEND (MELANOTRANSFERRIN-MTF, P97) A NEW VECTOR FOR DELIVERY OF BIOLOGICS ACROSS THE BBB FOR THE TREATMENT OF BRAIN DISEASES.

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biOasis Technologies Inc. is a biopharmaceutical company focused on the diagnosis and treatment of CNS diseases and disorders. The Company is developing a proprietary peptide vector (Transcend, MTf) for the delivery of therapeutics across the BBB.
Drug delivery into the CNS remains a significant challenge for clinical neuroscientists as most drugs show limited permeability across the brain capillary endothelium forming the BBB. We have developed p97 as a vector for drug delivery into the brain. The uptake is mediated by a receptor which has been related to the family of LDL Receptor related Protein (LRP). Here, we show that therapeutic drugs ''piggybacked'' as conjugates of MTf can be shuttled across the BBB for treatment of brain diseases.
MTf is very rapidly transported across the BBB into the brain parenchyma following intravenous injection as demonstrated by fluorescent derivatives of MTf conjugated molecules. Using MTf-cy5.5, we show by fluorescence microscopy that MTf conjugates are rapidly and efficiently transported in the brain parenchyma and in the lysosomal compartment in neurons and astrocytes. Using antibodies labeled with fluorescent molecules we demonstrated that antibodies against Her3 and against ßA1-42 peptides are transported across the BBB in brain cells after conjugation to MTf.
We have shown in previous experiments that aldurazyme (IDU) modified by incorporation of MTf can be transported across the BBB in MPS I mice (IDU-/-).A feasibility study has been started with Shire HGT in order to evaluate the application of biOasis platform technology Transcend (MTf) to transport lysosomal enzymes across the BBB in the lysosomal compartment of brain cells.
These studies provide the proof of concept that MTf can be used as a carrier capable of shuttling a variety of compounds ranging from small anti-cancer agent to larger biologics across the BBB into the brain parenchyma in therapeutic doses that enable treatment of neurological disorders.

ENHANCED BRAIN DELIVERY OF LIPOSOMAL METHYLPREDNISOLONE IS BENEFICIAL FOR THE TREATMENT OF NEUROINFLAMMATION

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Several promising drug candidates for the treatment of central nervous system (CNS) disorders are available. However, in order to achieve CNS effects they must be able to effectively cross the blood-brain barrier (BBB). The endogenous tripeptide glutathione (GSH) is found at high levels in the brain and is actively transported across the blood-brain barrier. Glutathione pegylated liposomes (G-Technology®) were found to mediate safe targeting and enhanced delivery of encapsulated drugs to the brain. Glutathione pegylated liposomal doxorubicin for patients with brain cancer is to-BBB's most advanced product; the first clinical trial in patients with brain metastases or primary brain tumors was initiated mid 2011.
The second most advanced product is glutathione pegylated liposomal methylprednisolone for the treatment of neuroinflammation. Neuroinflammation is associated with and contributes to a wide range of disorders of the CNS. An exploratory dose-range toxicity study showed that repeat administrations of therapeutically effective doses of glutathione pegylated liposomal methylprednisolone were well tolerated in contrast to similar doses of free drug, while the plasma circulation and brain uptake were significantly increased compared to free methylprednisolone. Furthermore, in an animal model of multiple sclerosis (MS), treatment using glutathione pegylated liposomes with methylprednisolone was significantly more effective compared to free methylprednisolone and non-targeted liposomes. Proof-of-concept studies in other neuroinflammatory disease models, including several models for lysosomal storage disorders, are currently ongoing.
In the past years to-BBB has gained much experience with the enhanced delivery of small molecules to the brain, yet, many more challenges lie ahead. to-BBB will continue to build the case for the G-Technology by extending preclinical research, thereby focusing on peptides, proteins, enzymes (e.g. for lysosomal storage disorders) and nucleic acids.

NOTES

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