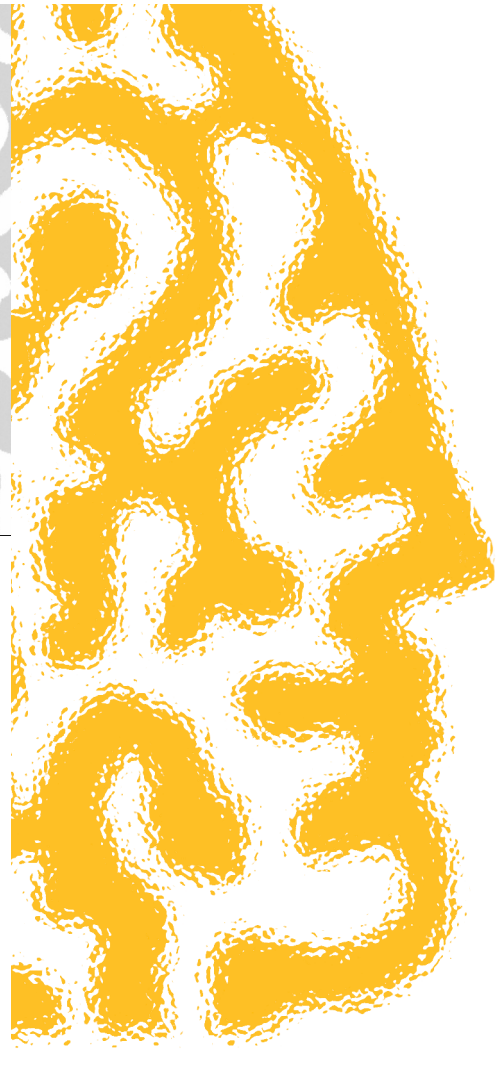

Fifth European Workshop

Frankfurt, Germany
Holiday Inn Hotel

March, 4th-6th 2011

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
www.brains4brain.eu info@brains4brain.eu

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 FOUNDATIONON

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 CHILDOHOOD: 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 Fifth Meeting of the Brains For Brain Foundation is following the first Gordon Conference Meeting on Lysosomal Diseases which was held in Galveston (Tx, USA) On January 23-28, 2011. The meeting was organised by two Brains For Brain Founding Members, Prof. Tony Futerman and Fran Platt. Prof. Futerman was the chair of the meeting.
Prof. Platt will chair the 2013 Gordon Conference on Lysosomal Diseases.
The Galveston meeting was outstanding and a lot of basic science was discussed.

The aims of this next workshop are:

1) to discuss research achievements in the field of neurodegenerative disorders at clinical and basic science level in the field of neurodegenerative lysosomal storage disorders and Blood Brain Barrier;
2) to discuss how B4B might collaborate with the European Union to stimulate interest in the research on LSDs and BBB. For this reasons representatives from EU Commission will be invited;
3) to discuss collaborations with international family associations and corporations to increase knowledge about storage diseases and research projects.

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

Scientific Officer
Cinzia Maria Bellettato (IT)

Logistics:
Jazz Travel & Congress, Spoleto, Italy - ph.+39 0743.221818 - p.caprelli@jazzitaly.com

NOTES

March 4th 2011

14.00-14.10
WELCOME AND OPENING

14.15-15.00
OPENING PLENARY LECTURE
PAUL SAFTIG, DE
New insights into lysosomal function.
University of Kiel.
Discussion.

15.00-19.15
BASIC ASPECTS AND BBB
Chair Discussants:
GERT FRICKER, DE - INGOLF BLASIG, DE

15.00-15.30
HELEN STOLP, UK
Effects of environmental influences on foetal cortex and vascular development.
University of Oxford.
Discussion.

15.40-16.10
BRITTA ENGELHARDT, CH
Migration of immune cells across the BBB.
Theodor Kocher Institute, Berne.
Discussion.

16.20-16.50
GIOVANNA DEL VECCHIO, IT
High throughput screening for claudin 5 modulators to manipulate the BBB.
Liebnitz Institute of Molecular Pharmacology, Berlin.
Discussion.

Coffee

17.20-17.50
ELGA DE VRIES, NL
BBB and multiple sclerosis: are there lessons for LSDs?
University of Amsterdam.
Discussion.

18.00-18.30
SANDRINE VITRY, FR
Cellular disorders in Mucopolysaccharidosis type III B.
Pasteur Institute, Paris.
Discussion.

18.40-19.10
ALESSANDRO FRALDI, IT
Cholesterol abnormalities and lysosomal dysfunction in LSDs.
TIGEM; Naples.
Discussion.

19.30 DINNER

March 5th 2011

9.00-13.30
PATHOPHYSIOLOGY AND LSDs
Chair Discussants:
ASHOK VELLODI, UK
VOLKMAR GIESELMANN, DE

9.00-9.30
MIA HORWITZ, IL
ER associated degradation and unfolded protein response in Gaucher disease.
University of Tel Aviv.
Discussion.

9.40-10.10
JON COOPER, UK
Batten disease.
Kings College, London.
Discussion.

10.20-10.45
EMYR LLOYD-EVANS, UK
Smith-Lemli-Opitz Syndrome; a recently identified lysosomal storage disease caused by defective cholesterol biosynthesis.
Cardiff University.
Discussion.

Coffee

CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 1

11.15-11.40
MARGARETA HAMMARLUND-UDENAES, SE
Drug delivery to the CNS.
University of Uppsala.
Discussion.

11.50-12.20
ROBERT KATONA, HU
A potential strategy for the treatment of neurological disorders: combined mammalian artificial chromosome-stem cell therapy.
Hungarian Academy of Sciences Szeged, Hungary
Discussion.

13.00 LUNCH

14.30-16.00
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 2
Chairperson:
JEAN MICHEL HEARD, FR
JÖRG KREUTER, DE

14.30-15.00
JÖRG KREUTER, DE
On the mechanism of nanoparticle drug delivery across the Blood-Brain Barrier: transport kinetics and influence of targeting igand attachment.
University of Frankfurt.
Discussion.

15.10-15.40
VOLKMAR GIESELMANN, DE
Transport of Arylsulfatase A across the Blood Brain Barrier.
University of Bonn.
Discussion.

15.50-16.20
ARI ZIMRAN, IL
OTC PC: From rats and type 1 Gaucher disease to patients with type 3 Gaucher disease: Give Ambroxol a chance!
Shaare Zedek Medical Center Jerusalem.

Coffee

17.00-17.30
BRIAN BIGGER, UK
The role of genistein in the treatment of MPSIII.
University of Manchester, UK.
Discussion.

17.40-18.10
SIMON WADDINGTON, UK
Perinatal gene therapy for lethal genetic diseases.
University College London, UK.
Discussion.

18.20-19.00
MARIE VANIER, FR
Plenary Lecture
Niemann-Pick C disease: the enigma and the challenges. *Inserm, FR.*
Discussion.

20.30 DINNER

March 6th 2011

B4B AND EUROPE

Chair Discussants:
MAURIZIO SCARPA, IT - DAVID BEGLEY, UK

8.45-9.20
B4B and Europe Activity report of activity and perspectives.

9.20-9.45
OLGA GOLUBNITSCHAJA, DE
Asphyxiated newborns - common origin but individual outcomes: time for new guidelines in personalised healthcare
EPMA, Brussels

9.45-10.15
LARS KRISTIANSEN, FR
Introduction to the ESF Forward Look on personalised medicine.
ESF, EMRC, Strasbourg, France.

10.15-10.45
General discussion.

Coffee

11.00-13.30
B4B AND BIOTECH COLLABORATIONS

Chair Discussants:
CATHERINE CAILLAUD, FR
MIA HORWITZ, IL

11.00-11.20
PERICLES CALIAS, USA
Intrathecal (IT) delivery of recombinant lysosomal enzymes.
Shire Human Genetic Therapies.
Discussion.

11.30-11.50
REINHARD GABATHULER, CA
A new peptide vector (p97) for delivery of therapeutic compounds across the BBB for the treatment of brain diseases.
biOasis Technologies
Discussion.

12.00-12.20
SEAN CLARK, USA
Improved pharmacological chaperones for the treatment of neuronopathic Gaucher and Parkinson's disease.
Amicus Therapeutics.
Discussion.

12.30-12.50
THOMAS KIRKEGAARD JENSEN, DK
Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated pathology.
Orphazyme
Danish Cancer Society, Denmark
Discussion.

13.00-13.30 LUNCH AND FAREWELL

NOTES

NEW INSIGHTS INTO LYSOSOMAL FUNCTION

PAUL SAFTIG
Biochemisches Institut, Christian-Albrechts Universität Kiel, Olshausenstr. 40, D-24098 Kiel, Germany;
e-mail: psaftig@biochem.uni-kiel.de

Lysosomes are the primary catabolic compartments of eukaryotic cells. These organelles are involved in various physiological processes, such as cholesterol homeostasis, antigen presentation, plasma membrane repair, bone and tissue remodelling, pathogen defence, cell death and cell signalling. Two classes of proteins are essential for lysosomal functioning; soluble lysosomal hydrolases and integral lysosomal membrane proteins. Each of the 50 known lysosomal hydrolases targets specific substrates for degradation, and their collective action is responsible for the total catabolic capacity in the lysosomal lumen. The importance of lysosomal trafficking pathways is emphasized by recent findings that reveal new roles for lysosomal membrane proteins in cellular physiology and in an increasing number of diseases that are characterized by defects in lysosome biogenesis. The mammalian lysosome contains at least 25 membrane proteins, but additional protein components of the lysosomal membrane are increasingly revealed. These proteins reside mainly in the lysosomal limiting membrane, which has a diversity of functions including acidification of the lysosomal lumen, protein import from the cytosol, sequestration of lysosomal hydrolases, mediation of fusion events, recruitment of molecular motor proteins and transport of degradation products to the cytoplasm. The major LMPs are the lysosome-associated membrane proteins (LAMP)-1 and -2, the lysosomal integral membrane protein (LIMP)-2 and the tetraspanin CD63. The presentation will provide an overview about the complex functions of the lysosomal compartment and the roles of lysosomal proteins in health and disease. Examples are provided which illustrate that the lysosome represents a central and dynamic organelle which is not simply the dead end of the endocytic pathway.

EFFECTS OF ENVIRONMENTAL INFLUENCES ON FOETAL CORTEX AND VASCULAR DEVELOPMENT

STOLP HB[1], DZIEGIELEWSKA KM[3], SAUNDERS NR[3], ANTHONY DC[2], MOLNAR Z[1].
Department of 1Physiology, Anatomy and Genetics, 2Department of Pharmacology, University of Oxford, United Kingdom, 3Department of Pharmacology, University of Melbourne, Australia.

Maternal inflammation during pregnancy is associated with damage to the foetal brain. Long-term behavioural deficits have been established, but the neuropathological basis for these deficits, and the role of the foetal brain barriers, is largely unstudied.
Systemic maternal inflammation was induced in pregnant C57Bl/6 mice by the injection of 500 or 10µg/kg lipopolysaccharide (LPS, E.coli O55:B5, Sigma) or saline at E13. A subset of animals was also injected with BrdU (Dako, 50mg/kg) immediately following the LPS injection. Animals were killed after 8h and foetuses were fixed by immersion in Bouin's fixative before being embedded in paraffin for sectioning. The structural integrity of the foetal brain was evaluated using immunohistochemistry for junctional protein and the status of the brain barriers was also assessed. The level of cell division in the ventricular zone was revealed with antibodies to phospho-Histone H3 and BrdU.
No changes were observed in the permeability of cortical vessels or the blood-CSF barrier to plasma protein in the foetus, following maternal inflammation, but the CSF-brain barrier became permeable to proteins from the cerebrospinal fluid in LPS treated animals. The presence of beta-catenin and claudin-5 proteins was altered at this barrier and there was a significant decrease in number of pH3 and BrdU positive dividing cells in the ventricular zone compared to saline treated controls.
Thus, we hypothesis that maternal inflammation may cause long-term behavioural changes in the offspring by of disrupting cell proliferation in the ventricle zone following changes in cerebrospinal fluid and the ventricular surface.

HIGH THROUGHPUT SCREENING FOR CLAUDIN-5 MODULATORS TO MANIPULATE THE BBB

DEL VECCHIO, G - TSCHEIK, C - NEWIE, I - BLASIG, IE.
Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany.

The blood brain barrier (BBB) limits the therapy of many CNS diseases. Furthermore, BBB is directly or indirectly compromised in many brain disorders. It is formed by endothelial cells sealed at the level of the paracellular cleft by tight junctions (TJ). Claudin-5 (Cld5) reduces the paracellular permeability of these TJ by acting as a size-selective filter for molecules <800 Da. Cld5 interacts in the plasma membrane of the same cell (homologous cis-interaction) and between plasma membranes of adjacent cells (homologous trans-interaction). The possibility to modulate the BBB via Cld5 could lead, on one hand to an improvement of CNS drug delivery, on the other hand to the re-establishment of barrier properties in CNS diseases whenever this feature is contributing or causing the pathological events. Cell-based High Throughput Screening (HTS) is based on imaging multiple targets in intact cells by analyzing multichannel fluorescence intensities. Here we describe our HTS approach to target Cld5 and identify small-molecule modulators of the BBB.
As first, we developed a cell-based HTS suitable for subcellular analysis of Cld5. Cld5-YFP was stably transfected in a TJ-free HEK-293 cells. The cell-cell contacts between two neighbouring cells expressing Cld5-YFP show enrichment of the YFP signal, due to the trans-interactions compared to the cell contacts between transfected and non-transfected cells (no enrichment, no trans-interactions). Counterstaining with a membrane marker allowed us to selectively detect the alterations of Cld5 at the contacts by following the YFP/membrane marker co-localization. For standardization of the assay, sodium caprate and Cld5 siRNA were used. They strongly decreased Cld5-YFP intensity at the cell-cell contacts in a transient, concentration and time dependent manner. Due to the multiple effects and treatment-induced variability, the statistical quality of this assay, as evaluated by the Z´-factor (maximum 0.25) resulted to be incompatible to the HTS. In order to improve the Z´-score and successfully transfer our assay to the HTS platform, we then introduced as control condition untransfected HEK cells and selected a simpler measurement parameter: the YFP intensity normalized against the cell number (nuclear staining). This revised assay and the consequent pilot screen provided a Z´-score ranging from 0.5 to 0.8, indicative of excellent analytical conditions enabling HTS of the complete small-molecule library. Thus, our assay targeting Cld5 to identify potential BBB opener and protective compounds was successfully implemented and adapted to the HTS technology.

THE BLOOD-BRAIN BARRIER AND MULTIPLE SCLEROSIS: ARE THERE LESSONS FOR LSDS?

ELGA DE VRIES
Department of Molecular Cell Biology and Immunology, MS center Amsterdam, VU medical center, Amsterdam, The Netherlands.
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In MS, a chronic inflammatory disorder of the central nervous system, an altered structure of the BBB has been described. Most studies focused on tight junction and basement membrane alterations and immune activation that lead to cellular influx, but data on the molecular properties of the BBB under neuroinflammatory conditions are scarce.
At the blood-brain barrier (BBB), the ATP binding cassette (ABC) transporters drive cellular exclusion of a variety of compounds, thereby protecting the brain from neurotoxic compounds. P-gp is a key ABC transporter located at the BBB, which effectively removes a remarkably wide variety of substrates out of the brain, including inflammatory agents. In using our well-defined post-mortem MS patient material, a dominant loss of P-glycoprotein (P-gp: ABCB1) was detected in active MS lesions (Kooij et al., J Autoimmun 2009:34:416-425). Strikingly, in MS lesions, reactive astrocytes start to express a number of these ABC transporters including multi-drug resistance protein-1 (MRP-1) and P-gp (Kooij et al., Brain 2010: 134:555-570).
Underlying mechanisms of an altered function of the BBB under inflammatory conditions remain unknown. However, in MS, the brain vasculature is surrounded by highly activated astrocytes (Van Doorn et al., Glia 2010: 58:1465-1476) that play a key role in the secretion of inflammatory mediators which may have an effect on the function of the BBB. Our recent results point towards an enhanced secretion of pro-inflammatory lipids such as ceramide by astrocytes towards the endothelium, which alters the function of the BBB.

Together, we here suggest that an altered interplay between activated astrocytes and the brain endothelium is an initial trigger of BBB dysfunction in MS, thereby allowing MS lesion formation.

CELLULAR DISORDERS IN MUCOPOLYSACCHARIDOSIS TYPE III B (MPSIIIB)

SANDRINE VITRY, ELISE ROY, THOMAS LEMONNIER, JULIE BRUYÈRE, STÉPHANIE BIGOU, STÉPHANE BLANCHARD, DELPHINE BOHL AND JEAN-MICHEL HEARD.

Children suffering from MPSIIIB, also known as Sanfilippo syndrome B, belong to the most severe subgroup of lysosomal storage diseases (LSDs) in terms of neurological deficits. In this syndrome, the lacking enzyme is the alpha-N-acetylglucosaminidase (NaGlu) which degrades heparan sulfate oligosaccharides (HSO) in the lysosomes. We have extensively studied the mouse model of MPSIIIB to decipher the links between HSO accumulation and pathological cascades. In MPSIIIB mouse cortical neurons, vacuolation, which is a hallmark of LSDs, occurred in the absence of major alterations of endocytosis or macro-autophagy. Interestingly, LAMP1-positive vacuoles contained the cis-Golgi matrix protein GM130 in their limiting membrane. Electron microscopy suggested the involvement of the Golgi apparatus in vesicular distensions. Fluorescent microscopy showed excessive extension of GM130-positive Golgi ribbons in MPSIIIB neuronal prolongations. Brefeldin A did not completely disaggregate Golgi structures and had no effect on GM130/LAMP1 double-positive vesicles.
We have created two NaGlu-deficient cellular models to further investigate the role of GM130 in the pathogenic cascade induced by HSO accumulation. We generated induced pluripotent stem cells (iPSc) from skin fibroblasts of Sanfilippo B children to get access to human affected neurons. Both patient iPSc and derived neurons developed typical storage vesicles with LAMP1 and GM130 in their limiting membrane and showed severe disorganization of Golgi ribbons. We also generated inducible HeLa cells in which NaGlu expression is inhibited through the expression of specific shRNAs. In contrast to chronically deficient iPSc and neurons, this inducible system allows establishing a chronology of early pathological events. NaGlu-depleted HeLa cells recapitulated previously described vacuolation and Golgi defects. They also developed an acute pathology characterized by GM130 association to cytoskeletal elements, increased microtubule stability, centrosome multiplication and defective cell division. With regards to gene expression profiling, we are currently exploring Golgi-dependent responses to extracellular signals which may be altered in MPSIIIB.

ER ASSOCIATED DEGRADATION AND UNFOLDED PROTEIN RESPONSE IN GAUCHER DISEASE

IDIT RON, INNA BENDIKOV-BARR, HEDVA SHMERLING AND MIA HOROWITZ
Department of Cell Research and Immunology, Tel Aviv University, Israel.

Mutations in the glucocerebrosidase-encoding gene, GBA1, lead to accumulation of gucosyl-ceramides, manifested as Gaucher disease. We have shown that mutant glucocerebrosidase variants present variable degrees of ER retention and undergo ER associated degradation (ERAD) in the proteasome. The ERAD process requires specific E3 ligases, which ubiquitinate the misfolded enzyme before its elimination in the proteasome. Gaucher mutations have been recently identified as a major cause for Parkinson disease. One of the genes associated with Parkinson disease is PARK2, encoding an E3 ligase. Based on these observations, we tested the possibility that the concurrence of GD and PD reflects an association between parkin and misfolded mutant glucocerebrosidase variants. Our results strongly indicated that several glucocerebrosidase variants undergo parkin mediated K48 ubiquitination and degradation in the proteasome. In the presence of proteasome inhibition, parkin promoted aggregation of mutant glucocerebrosidase.
ERAD, as well as the productive folding mechanism, is induced in response to ER stress, an imbalance between the load of unfolded proteins that enter the ER and the capacity of the cellular machinery that handles this load sets. These are two processes regulated by a transcriptional program termed the unfolded protein response (UPR). leading to degradation of unfolded proteins and accelerated refolding. UPR is associated with a change in gene expression, and, therefore, can be followed by testing the RNA levels of several key genes, involved in this process. We tested UPR in 12 different GD derived skin fibroblasts. In a major part of the studied samples there was UPR, manifested by increase in BiP or CHOP and splicing of XBP1. However, there was no direct correlation between the level of UPR and GD severity.

SMITH-LEMLI-OPITZ SYNDROME, A RECENTLY IDENTIFIED LYSOSOMAL STORAGE DISEASE CAUSED BY DEFECTIVE CHOLESTEROL BIOSYNTHESIS

EMYR LLOYD-EVANS
School of Biosciences, Prifysgol Caerdydd, Heol yr Amgueddfa, Caerdydd, CF10 3AX, UK.

Smith-Lemli-Opitz syndrome (SLOS) is an inherited metabolic disease in which the endoplasmic reticulum (ER) cholesterol biosynthetic enzyme (3β-hydroxysterol Δ7-reductase) is defective. This causes accumulation of the cholesterol precursor, 7-dehydrocholesterol (7-DHC). A potential therapy for SLOS patients is to increase dietary cholesterol intake, surprisingly this is of limited clinical benefit. Recent work has shown that 7-DHC accumulation in SLOS cells leads to defective transport of exogenous LDL-derived cholesterol from late endosomes to the ER where it is normally utilised. This causes free cholesterol storage in late endosomes, mimicking another lipid transport disorder, Niemann-Pick disease type C1 (NPC1). Thus, the reason SLOS patients cannot correctly utilize dietary cholesterol is that it is not transported to the ER.

7-DHC is a secondary amphiphile, a group of molecules capable of inducing an NPC1-like cellular lysosomal storage phenotype. We have discovered that accumulation of 7-DHC, which potentially interferes with NPC1 protein function, in SLOS cells and tissues leads to lysosomal storage of multiple lipids. These include sphingosine, which ultimately inhibits cholesterol efflux from lysosomes to the ER. Our findings point towards a regulatory role of sterols in maintaining correct NPC1 function but also indicate that it is sphingolipids and not sterols that cause the endocytic dysfunction seen in NPC1 disease.

Treating SLOS cells with NB-DGJ, to lower glycosphingolipid biosynthesis (reducing sphingolipid storage), clears lysosomal storage bodies and corrects the SLOS lysosomal transport defect resulting in delivery of LDL-cholesterol to the ER for utilisation. SLOS patients may benefit from glycosphingolipid lowering drugs in combination with dietary cholesterol elevation.

We therefore propose that SLOS is a ‘secondary’ lysosomal storage disease caused by the accumulation of a metabolite, 7-DHC, directly linked to the primary metabolic defect, ER cholesterol biosynthesis

DRUG DELIVERY TO THE CNS

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Predicting drug delivery to the brain is complicated because of the specific functions of the blood-brain barrier (BBB) with its active efflux and influx transporters. It is likely that there are still many undiscovered transporters at the BBB that affect currently used drugs. Also, one drug can be a substrate for several transporters. In vivo investigations are therefore essential to clarify how the combined transporter functions promote or restrict drug delivery to the brain. The presentation will be based on relationships for unbound brain concentrations, here defined as the drug concentrations in brain interstitial fluid (ISF), as these are better related to pharmacologically active concentrations at the target site than the total brain concentrations.

Three aspects can be used to describe brain drug delivery, the rate of delivery, the extent of delivery of unbound drug, and the affinity of the drug for brain tissue. Their respective importance for predicting clinical success will be presented and put into context with total brain concentrations. The role of CSF measurements for brain ISF predictions will also be discussed, as well as other methods of importance for understanding brain drug delivery, among them PET for studying species differences in transporter function and predictability of rodent data for the human situation. Chemical properties of drugs in relation to brain penetration will also be included.

ON THE MECHANISM OF NANOPARTICLE DRUG DELIEVER ACROSS THE BLOOD BRAIN BARRIER: TRANSPORT KINETICS AND INFLUENCE OF TARGETING LIGAND ATTACHMENT

JÖRG KREUTER
Institut Für Pharmazeutische Technologie, Goethe-Universität Frankfurt, Germany.

The blood-brain barrier (BBB) restricts or prohibits the transport of most drugs from the blood circulation into the brain. Nanoparticles made of poly(butyl cyanoacrylate), human serum albumin (HSA), polylactic acid (PLA), or poly(lactide-co-glycolide) (PLGA) can enable the transport of such drugs across the BBB after overcoating with certain surfactants such as polysorbate 80 or poloxamers 188 or by covalent attachment of certain targeting ligands. Drugs that have been transported across this barrier and exhibited significant pharmacological effects in the brain included the cytostatic drug doxorubicin, nerve growth factor (NGF) with a molecular weight of about 130 kDa, and others.

The kinetics of the doxorubicin transport across the BBB was evaluated in rats by the capillary depletion method which demonstrated that significant amounts of this drug were transported into the brain by the polysorbate 80-coated PBCA nanoparticles. After centrifugation of the brain homogenates with this formulation 2 h after i. v. injection the amounts of doxorubicin in the supernatant representing the brain parenchyma were double as high as in the pellet which consists mainly of the blood vessels and cell debris including cell nuclei.

An analysis of the nanoparticle transport mechanism by transmission electron microscopy indicated that the mechanism appears to be receptor-mediated endocytosis followed by transcytosis. No opening of the tight junctions was observed. By overcoating with polysorbate 80 or poloxamers 188 depending on the core polymer apolipoproteins A-1 and/or E will be adsorbed in the blood on to the nanoparticle surface after i.v. injection. These apolipoproteins then lead to the interaction with LDL or scavenger receptors on the brain capillary endothelial cells followed by above processes. Likewise, the covalent attachment of these apolipoproteins or of transferrin, insulin, or antibodies against the transferrin or insulin receptors also enables a similar nanoparticle-mediated drug transport across the BBB. These nanoparticles obviously act as Trojan Horses.

TRANSPORT OF ARYLSULFATASE A ACROSS THE BLOOD BRAIN BARRIER.

VOLKMAR GIESELMANN, ULLRICH MATZNER, FRANK MATTHES, ANNIKA BÖCKENHOFF
Institute for Biochemistry and Molecular Biology, University of Bonn, Germany.

HANS JOACHIM GALLA, SABINE HÜWEL, MAREIKE SCHULZ, PHILIPP WÖLTE
Institute for Biochemistry, University of Münster, Germany.

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder caused by the deficiency of arylsulfatatase A (ASA). This leads to a block in the degradation of sulfatide, a major myelin lipid and causes progressive finally lethal demyelination in patients. High dose enzyme replacement trials in a mouse model of the disease surprisingly showed a reduction of sulfatide accumulation in the brain suggesting that a small amount of enzyme may have passed the blood brain barrier (BBB). Indeed, when endothelial cells and brain parenchymal cells were separated after enzyme injection, small amounts of enzyme were found in the brain cell fraction. Using an in vitro blood brain barrier cell culture model based on primary bovine cells, we could show that a small amount of ASA does pass the blood brain barrier in this in vitro model. Mannose-6-phosphate dependent transcytosis contributes little to this transfer, most of it is due to adsorptive binding of the enzyme to cells and subsequent non-receptor-mediated transcytosis. Thus, as a consequence cationization and an increased content of mannsoe-6-phosphate may increase the delivery of enzyme across the BBB in vivo.

OTC PC: FROM RATS AND TYPE 1 GAUCHER DISEASE TO PATIENTS WITH TYPE 3 GAUCHER DISEASE: GIVE AMBROXOL A CHANCE!

ARI ZIMRAN
Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem Israel.

Enzyme replacement therapy has revolutionized management of Gaucher disease (GD) yet due to its inability to cross the blood-brain-barrier (BBB) it cannot prevent deterioration nor to reverse the neurological features of type 3 (GD3).
A clinical trial in GD3 using miglustat (Zavesca; Actelion Pharamceuticals) has failed because of inability to meet end-points, while the pharmacological chaperone (PC) isofagomine (Plicera, Amicus Therapeutics Inc) which had a potential to cross the BBB, also failed phase 2. The on-going clinical trials of eliglustat (Genzyme Corporation) are restricted to GD1, so at present there are not any trials for GD3.
We herein propose a clinical trial using Ambroxol hydrochloride, a mucolytic agent which is available over-the-counter in many countries. Ambroxol has been identified by Don Mahuran's group (Toronto Canada) as PC for GD with some potential advantages over isofagomine. Ambroxol has been shown to enhance in-vitro enzymatic activity of various mutated glucocerebrosidase enzymes, including that of the N370S mutation and the L444P, the latter is the most prevalent mutation in GD3. Previous animal studies provided hints that Ambroxol may pass the BBB and hence the potential value in neuronopathic forms.
We have administered "off-label" Ambroxol to 12 patients with mild GD1, where only the skinniest patient had good results, suggesting the need for higher doses.
Our proposal involves one year where safety and dose finding will be assessed in GD1 patient and further studies in rats for BBB penetration. Based on those results second and third years will focus on GD3 with the primary end-point of neuro-cognitive functions as assessed by the MindStreams® computerized battery.
The unfortunate issue with Ambroxol is that it is cheap and widely available, and therefore unattractive for investors. It is for this reason that we are hoping that the academicians and patient advocacy groups will give Ambroxol a chance!

THE ROLE OF GENISTEIN IN THE TREATMENT OF MPSIII

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The tyrosine kinase inhibitor genistein aglycone is widely available as a food supplement, occurring naturally within soy foods, predominantly in a glycoside form (genistin) and can also be synthesised or purified in its aglycone form.
Genistein aglycone has been shown to reduce glycosaminoglycan (GAG) substrate accumulation in fibroblasts of several mucopolysaccharide diseases including MPSIIIA, B and C. Subsequently, a concentrated supplement form of soy extract (Soyfem) has been used in clinical trial for patients with MPSIIIA and IIIB at 5mg/kg/day, doses that we would predict from our 2009 data in the mouse model of MPSIIIB would not be effective in the brain but may clear peripheral storage. This open label study was not designed to study neurological outcomes but did show small reductions in urinary GAGs. A clinical trial for patients with MPS IIIA (a clinically indistinguishable disorder) is underway in the Netherlands but this also uses a low dose of genistein (10 mg/kg/day) in supplement form (Soyfem) and data is not yet available from this trial.
Genistein aglycone has low oral toxicity in mammals and around 10% blood brain barrier permeability. However, genistein aglycone is reported to be less susceptible to degradation by gut flora than genistin and has higher plasma bioavailability than mixtures of genistein/genistin in concentrated soy extract such as Soyfem. Thus it is possible that Soyfem will not be as effective as genistein aglycone at the same dose.

We have previously shown that short-term administration of doses up to 160 mg/kg/day genistein aglycone significantly reduces liver lysosomal storage in mice with MPSIIIB and present here data on long-term administration of the same 160 mg/kg/day dose which significantly reduces brain lysosomal storage by a third, neuroinflammation by at least a sixth, delays synaptic loss and corrects behaviour in mice with MPSIIIB. No measurable toxicity was observed in these mice.
The doses used in our preclinical work far exceed any used in patients to date and are higher again than those used in a murine model of MPSII which used Soyfem supplementation. Despite the concerns over dosing, some US MPSIII patients have managed to obtain pure genistein aglycone and begin daily supplementation at doses of 100 mg/kg/day. We have posted a position statement on genistein on the Manchester website encouraging patients to wait for the outcomes of a definitive double blinded placebo controlled clinical trial in MPS III that we plan to run in a year's time in Manchester, subject to funding, to determine if high doses of synthetic genistein aglycone will be effective and safe in patients or not.
A definitive answer to this question is critical to the welfare of MPSIII patients.

PERINATAL GENE THERAPY FOR LETHAL GENETIC DISEASES

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Several inherited neurological diseases are characterised by neurodegenerative changes at or around birth. Prognosis remains dismal and for several of these diseases, including acute neuronopathic Gaucher Disease, there is no treatment and palliative care remains the only option. Fetal or neonatal gene therapy may provide a means of pre-empting the rapid onset of neurological damage in these diseases. Recently, AAV9 has been shown by others to transduce the brain and spinal cord of mice, cats and macaques following intravenous injection. We have investigated this further by comparing gene expression after intravenous injection into fetal and neonatal mice. AAV9 vector driving green fluorescent protein under the cytomegalovirus (CMV) promoter was injected into the fetal mouse circulation via the vitelline vessel at 16 days post-conception. GFP expression was observed in neurons throughout the CNS. In contrast, neonatal administration resulted in expression predominantly in protoplasmic astrocytes, as verified by scanning confocal microscopy. Extensive GFP expression was observed, and quantified, in visceral organs, muscle, bone, eye, and skin and the peripheral nervous system. When GFP was driven by a truncated β-glucuronidase promoter rather than CMV, expression was restricted to neurons. Administration of AAV9-CMV-GFP to the circulation of fetal macaques by ultrasound-guided injection resulted in extensive transduction of nerves throughout the central and peripheral nervous system. AAV9 may, therefore, serve as a useful tool for dissection of the role of different cells and tissues underlying the pathogenesis of disease models of neurodegenerative disease. Furthermore they may provide a pathway towards therapeutic intervention.

NIEMANN-PICK C DISEASE : THE ENIGMA AND THE CHALLENGES

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Among lysosomal neurolipidoses, Niemann-Pick C disease (NP-C) is atypical in many ways. It is caused by defects in either of two late endosomal/ lysosomal proteins (transmembrane NPC1 or soluble NPC2) shown to function in a nonredundant coordinate fashion. In extraneural tissues and cells, the most obvious metabolic lesion involves a unique impairment in processing and utilization of endocytosed cholesterol. This alone might explain the complex lipid storage observed in patients and animal models, although challenging hypotheses have been proposed. This alteration is used for laboratory diagnosis of patients but it shows in cultured cells wide variations depending on the mutation. Variant phenotypes with minimal cholesterol transport abnormalities have not been thoroughly investigated and no such animal model has been generated. In spite of recent elegant studies focusing on cholesterol transport, the precise function(s) of the NPC2 and above all NPC1 proteins are still largely unknown. To add to the complexity, the situation is particularly obscure in the brain, where neurons accumulate mostly GM2 and GM3 gangliosides, and much less cholesterol (or free sphingoid bases). What causes the prominent but patterned neuronal loss and other neuropathological changes remains an enigma. Most experimental studies have been conducted in mesenchymal cells which may have obscured abnormalities occurring in neuronal cells. Visceral manifestations can be prominent in early life, but NP-C is primarily a degenerative neurological disease, with an incidence of ~ 1/100 000 births. Close to 95% of patients have NPC1 mutations. Due to the properties of the NPC1 protein, several therapeutic approaches followed for “classical” lysosomal storage diseases are not applicable. Miglustat is currently the only approved disease-modifying drug. 2-Hydroxypropyl-ß-cyclodextrin currently appears as the most effective experimental therapy, but its mode of action is not well understood, and differences are observed between the mouse and the cat models. Understanding NP-C remains a formidable challenge.

ASPHYXIATED NEWBORNS - COMMON ORIGIN BUT INDIVIDUAL OUTCOMES: TIME FOR NEW GUIDELINES IN PERSONALISED HEALTHCARE

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Clinical observations clearly demonstrate that similar endogenous and exogenous risk factors cause individual reactions and pathologic characteristics; therefore, the same therapeutic approaches applied within one cohort of patients lead to individual outcomes.

The most frequent pathology in newborns is a perinatal asphyxia – mild or severe oxygen deficiency that is registered for 1-100 from 1000 life births depending on country of origin. Insufficient oxygen supply causes ischemic organ damage in newborns followed by either a fatal outcome or mild/severe life-long pathologies. Increased morbidity and mortality, hypoxic-ischemic encephalopathy, mental retardation, neurodegenerative diseases, nephropathy, cardiomyopathy, vascular pathologies, senescence, diabetes mellitus and cancer all belong to the short- and long-term outcomes of birth asphyxia. The task of individual prediction and well-timed targeted prevention of the life-long chronic pathologies usually developed by asphyxiated newborns should be given the extraordinary priority in pediatrics.

Individualised treatment algorithms and paradigm change from a late interventional approach to predictive diagnostics followed by the targeted prevention of a disease before it manifests, presents an innovative concept for advanced healthcare that is costs effective. Predictive perinatal / postnatal diagnostics and the pre-selection of healthy but disease-predisposed individuals, followed by targeted preventive measures, represent the primary task in the overall action of personalised healthcare. Those highly effective measures can lead to a reduced prevalence of severe pathologies and better long-term outcomes for patients treated according to individual parameters and therapeutic algorithms. Furthermore, increased portion of socially active members remaining vibrant with excellent physical and mental health can therefore, be expected in the elderly. Improving the quality of life of aging populations and reducing costs in advanced healthcare systems, is a global challenge of 21st century. This task requires intelligent political regulations and creation of new guidelines to advance the current healthcare systems. Targeted preventive measures should be well regulated by innovative reimbursement programmes introduced by policy-makers. This is considered as the cost-effective preventive "medicine of the future". The overall concept in the field is conducted by the "European Association for Predictive, Preventive and Personalised Medicine" (EPMA).

INTRODUCTION TO THE ESF FORWARD LOOK ON PERSONALISED MEDICINE

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The European Science Foundation (ESF; www.esf.org) is an independent non-governmental organisation funded by its Member Organisations, European funding and performing organisations as well as scientific academies. The ESF's aim is to act as a catalyst for the development of science by bringing together leading scientists and research funding agencies to debate, plan and implement pan-European initiatives and to explore new directions for research at the European level.
Healthcare is on the brink of a revolution precipitated by dramatic advances in biomedical research. The ability to distinguish, at the molecular level, what makes one person different from another lies at the heart of this fundamental shift. Combined, these developments will change our approach to medicine from finding cures towards individualised prediction, diagnosis, treatment and prevention. Indeed, individualised biological profiles will in the future be used to determine a person's individual healthcare needs. This paradigm shift has been coined as 'personalised medicine'.
The European Medical Research Councils (EMRC), which is the membership organisation for all the medical research councils in Europe under the ESF, has decided to conduct a foresight initiative termed 'Personalised Medicine for the European Citizen'. This field represents an important strategic priority area that involves not only biomedical and technological issues, but also impinges on overarching societal, ethical, economical and legal questions, which is why this Forward Look is supported by all five ESF Standing Committees.
Forward Looks are ESF strategic foresight instruments, intended to enable Europe's scientific community, in interaction with policy makers, to develop medium to long-term views and analysis of future research developments with the aim of defining research agendas at national and European level. The present presentation will introduce the Forward Look instrument and outline the priority areas of this initiative.

INTRATHECAL (IT) DELIVERY OF RECOMBINANT LYSOSOMAL ENZYMES

PERRY CALIAS, PH.D
Shire HGT.

Lysosomal storage diseases (LSDs) are a family of genetic disorders caused by missing or defective enzymes, resulting in abnormal substrate accumulation. While the peripheral symptoms associated with several of these diseases can be effectively mitigated by intravenous administration of recombinant enzymes, they are not expected to significantly impact the CNS manifestations associated with a majority of the LSDs. We have therefore undertaken a program to investigate the intrathecal delivery of recombinant human arylsufatase (rhASA) and an IT specific formulation of idursulfase (idursulfase-IT).
Repeat monthly administration of idursulfase-IT and rhASA to Cynomolgus monkeys for six months was generally well tolerated and not associated with any significant adverse toxicologic events.
Both Idursulfase and rhASA was detected in all areas of the brain by immunohistochemistry, with a deposition gradient from the cerebral cortex to the ventricular white matter. Co-localization staining revealed that IT administration of both enzymes is associated with both neurons and oligodendrocytes. Additionally, administered enzyme appears to be associated with axonal structures, suggestive of anterograde axonal transport of Idursulfase-IT and rhASA.
Studies evaluating 3 weekly IT administrations of Idursulfase-IT in the Hunter IKO mouse demonstrated a marked reduction in CNS cellular vacuolization at both light and electron microscopic levels and in lysosomal disease pathological biomarker, lysosomal associated membrane protein 1. Normalization of abnormal lysosomal function correlated with dramatic morphological improvements in all areas of the brain.

Taken together, this series of experiments demonstrates that IT administration of Idursulfase-IT and rhASA is generally well tolerated, reaches the target CNS tissues, and is efficacious in the appropriate animal disease model for the CNS components of Hunter syndrome.

A NEW PEPTIDE VECTOR (P97) FOR DELIVERY OF THERAPEUTIC COMPOUNDS ACROSS THE BBB FOR THE TREATMENT OF BRAIN DISEASES

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biOasis Technologies Inc. is abiopharmaceutical company focused on the diagnosis and treatment of CNS diseases and disorders. The Company is developing a test for diagnosis of Alzheimer's disease plus a proprietary peptide vector 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 blood-brain barrier (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 p97 can be shuttled across the BBB for treatment of brain diseases.
p97 is very rapidly transported across the BBB into the brain parenchyma following intravenous injection as demonstrated using radiolabeled, gold and fluorescent derivatives of p97 conjugated molecules. Our work with p97 shows that brain levels ofradioiodinated p97 in mice were up to 7-10% of the injected dose per body weight 1 hour after IV injection. Using p97-cy5.5 and p97-gold we show by fluorescence microscopy and electron microscopy that p97 conjugates are rapidly and efficiently transported in the brain parenchyma. Therapeutic doses of doxorubicin are transported to the brain after conjugation to p97 as measured by the increase of survival of mice intracranially implanted with glioma. p97ADR (40%). Doxo alone did not improve survival. Additionally we show that aldurazyme (IDU) modified by incorporation of p97 can be transported across the BBB in MPS I mice (IDU-/-).
This study provides the initial proof of concept that p97 can be used as a carrier capable of shuttling a variety of compounds ranging from small anti-cancer agent to larger biologics such as enzymes across the BBBinto the brain parenchyma in therapeutic doses that enable treatment of neurological disorders.

IMPROVED PHARMACOLOGICAL CHAPERONES FOR THE TREATMENT OF NEURONOPATHIC GAUCHER AND PARKINSON'S DISEASE

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Gaucher disease and Parkinson's disease (PD) may share an etiological basis because GBA1 mutations, which lead to a deficiency of acid beta-glucosidase (GCase) activity and Gaucher disease when homozygous, are also associated with increased risk for PD when either hetero- or homozygous. Neuronopathic forms of Gaucher disease and PD also share the therapeutic challenge of targeting the central nervous system (CNS). Pharmacological chaperones (PC) are orally-available, small molecules that represent an innovative approach to specifically increase the quantity and stability of target proteins. AT2101 is a PC that was developed to target GCase. Administration of AT2101 to murine models of PD increased endogenous levels of brain GCase and importantly, prevented the accumulation of a-synuclein, a hallmark of this disease.

We reasoned that the CNS exposure and other properties of AT2101 could be further improved while maintaining a satisfactory safety profile. For instance, AT2101 interacts with targets other than GCase, such as glycogen phosphorylase, and its EC50 for GCase enhancement is greater than the Ki for inhibition of GCase at lysosomal pH. Although AT2101 has a pharmacodynamic effect on brain GCase, exposure in the CNS is only a fraction of that seen in the plasma. AT2101 also has relatively long tissue and lysosomal half-lives.

An assessment of nearly 200 analogs of AT2101 led to the identification of several new PCs with superior characteristics, including greater CNS penetration, increased potency for enhancement of GCase activity, and improved target specificity. Another important property of these analogs is the difference between their EC50 values for GCase enhancement and their IC50 values for GCase inhibition in the lysosome. For several of these analogs, the EC50 values are much lower than their in situ lysosomal IC50 values, providing a potential window for GCase enhancement with less interference from inhibition. Lastly, many of the analogs have accelerated efflux from both tissues and lysosomes. In conjunction with the EC50/IC50 difference, this property expands the interval during which the enhanced GCase operates unimpeded by inhibition. These improved PCs are currently under investigation in models of PD and neuronopathic Gaucher disease.

HSP70 STABILIZES LYSOSOMES AND REVERTS NIEMANN-PICK DISEASE-ASSOCIATED PATHOLOGY

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Heat shock protein 70 (Hsp70) is an evolutionarily highly conserved molecular chaperone that promotes the survival of stressed cells by inhibiting lysosomal membrane permeabilization.
Here we show that Hsp70 stabilizes lysosomes by binding to bis(monoacylglycero)phosphate (BMP), an essential co-factor for lysosomal sphingomyelin metabolism. In acidic environments Hsp70 binds with high affinity and specificity to BMP, thereby facilitating the BMP binding and activity of acid sphingomyelinase (ASM). The inhibition of the Hsp70–BMP interaction by BMP antibodies or a point mutation in Hsp70 (Trp90Phe), as well as the pharmacological and genetic inhibition of ASM, effectively revert the Hsp70-mediated stabilization of lysosomes. Notably, the reduced ASM activity in cells from patients with Niemann–Pick disease A and B is also associated with a marked decrease in lysosomal stability, and this phenotype can be effectively corrected by treatment with recombinant Hsp70. Taken together, these data open exciting possibilities for the development of new treatments for lysosomal storage disorders.1

1) Kirkegaard et al, Nature. 2010 Jan 28;463(7280):549-553.

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