Seventh European Workshop

Frankfurt, Germany
Holiday Inn Hotel

March 8th-10th, 2013

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.
Since lysosomal enzymes are 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 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 was 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, 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 Seventh Meeting of the Brains For Brain Foundation are:

- 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;
- to discuss new recent advances on natural history and pathophysiology of LSDs 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 disease;
- 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;
- 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.

PHD MEETING

Brains for Brain Foundation, together with NCL-Foundation is organizing a Joint PhD meeting to be held in Frankfurt on March 6-7, 2013. The meeting is targeted to young scientists that will group together for sharing experiences and ideas

The meeting has the following main objectives:

- To bring together the best and most deserving PhD students
- To allow them to present their work in a relaxed and constructive environment
- To facilitate the exchange of dissertation research studies on Rare diseases and in particular on LSDs
- To prepare PhD students for a successful career on LSDs field
- To allow students to built relationship with one of the most important European network of Internationally renewed experts working on LSDs

Moreover the meeting will offer PhD students the chance to participate and present their work at the 7th B4B meeting.

The NCL-Foundation is a non-profit organisation located in Hamburg, Germany that is dedicated to Batten disease. The foundation lists its principal goals as:
- Increasing public awareness of NCL in order to promote the early diagnosis of the disease.
- Building an NCL network of medical specialists and basic science researchers of different disciplines, in order to coordinate national and international expertise.
- Initiating research to develop possible cures.

The NCL-Foundation is committed to cooperative research funding. To initiate important NCL research projects, the Foundation is continuously on the lookout for suitable cooperation partners from both the profit and non-profit sectors.

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

Scientific Officer
Cinzia Maria Bellettato (IT)

Logistics
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March 8th 2013

14.00-14.10
WELCOME AND OPENING

14.15-15.00
OPENING PLENARY LECTURE
L. DREWES, *University of Minnesota, USA*
Searching for chinks in the armor: Perspectives on the neurovascular unit and delivery of CNS therapeutics

15.00-16.45
BASIC ASPECTS
Chair Discussants:
M. VANIER, F - V. GIESELMANN, D

15.00-15.25
N. J. BUCKLEY, *Kings College London, UK*
Neurodegeneration as an RNA disorder
Discussion.

15.35-16.00
M. GEGG, *University College London, UK*
Glucocerebrosidase deficiency and Parkinson's disease
Discussion.

16.10-16.35
M. HORWITZ, *Tel Aviv University, Israel*
The association between Gaucher disease and Parkinson disease: from Human to Drosophila
Discussion.

Coffee

17.00-17.25
D. E. SLEAT, *Center for Advanced Biotechnology and Medicine, New Jersey, USA*
Proteomic analysis of mouse models of Niemann-Pick C disease reveals alterations in the steady-state levels of lysosomal proteins within the brain
Discussion.

17.35-18.00
E. LLOYD EVANS, *University of Cardiff, UK*
Intra-lysosomal accumulation of free Zn^2+ in Niemann-Pick disease type C1 correlates to neuronal loss and can be treated with chelators
Discussion.

19.30 DINNER

March 9th 2013

9.00-13.30
PATHOPHYSIOLOGY AND LSDs
Chair Discussants:
M. HORWITZ, IL - E. LLOYD EVANS, UK

9.00-9.25
C. DOTTI, *University of Madrid, Spain*
Role of membrane cholesterol in neuronal survival and plasticity in the old brain
Discussion.

9.35-10.00
B. BOLAND, *University College Cork, Ireland*
Neuronal hallmarks of impaired lysosomal flux: Relevance to lysosomal storage diseases and Alzheimer's disease
Discussion.

10.10-10.35
B. BIGGER, *University of Manchester, UK*
Neuroinflammation in LSDs - cause or consequence?
Discussion.

Coffee

11.00-12.45
PATHOPHYSIOLOGY AND LSDs 2
Chair Discussants:
B. BIGGER, UK - D. ZAFEIRIOU, GR

11.00-11.25
T. FARFEL-BECKER, *Weiztmann Institute, Israel*
The pathogenesis of neuronopathic Gaucher disease
Discussion.

11.35-12.00
J. M. HEARD, *Pasteur Institute Paris, France*
Cellular and molecular pathology in Sanfilippo syndrome
Discussion.

12.10-13.00
CNL PhD Students

13.00 LUNCH

14.00-16.20
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTIC OPTIONS
Chair Discussants:
J. M. HEARD, F - I. BLASIG, D

14.00-14.25
A. FRALDI, *TIGEM, Italy*
A highly secreted sulfamidase engineered to cross the blood-brain barrier corrects the CNS pathology of mice with mucopolysaccharidoses type IIIA
Discussion.

14.35-15.00
B. BANKS, *University of Washington, USA*
Transport of lysosomal enzymes and HIV-1 from blood to brain by the M6P receptor: effects of neuroinflammation and adrenergic regulation
Discussion.

15.10-15.35
A. PIETROIUSTI, *University of Rome, Italy*
Interactions of engineered nanoparticles with organs protected by internal biological barriers
Discussion.

15.45-16.10
M. DELI, *Hungarian Academy of Sciences, Hungary*
Opening intercellular junctions of brain endothelial cells with peptides
Discussion.

Coffee

16.45-19.30
CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTIC OPTIONS 2
Chair Discussants:
J. KREUTER, D - D. BEGLEY, UK

16.45-17.10
K. DAWSON, *University College Dublin, Ireland*
Targeting nanoparticles to the CNS: The role of the protein corona
Discussion.

17.20-17.45
S. C. GARMAN, *University of Massachusetts, USA*
Pharmacological chaperones for human α-N-acetylgalactosaminidase
Discussion.

18.00-18.45
PLENARY LECTURE
K. HALDAR, *University of Notre Dame, IN USA*
Genomic expression analyses reveal lysosomal, innate immunity proteins, as disease correlates in murine models of a lysosomal storage disorder
Discussion.

20.30 DINNER

March 10th 2013

08.30-11.00
B4B AND EUROPEAN ACTIONS
Chair Discussants:
D. BEGLEY, UK - M. SCARPA, I

8.45-9.10
M. ENSINI, *EURORDIS*
Research on Rare Diseases: international and patient inclusive
Discussion.

9.20-9.45
M. SCARPA, *Brains For Brain Foundation, EU*
Presentation of the Inherited NeuRoMetabolic Diseases Information Network (InNerMed-INetwork)
Discussion.

9.45-10.10
J. GREEN, *President of the International Niemann Pick Alliance*
An EU rare diseases registry for Niemann Pick Disease type A,B and C
Discussion.

10.25-10.50
V. GIESELMANN, *University of Bonn, G, TBC*
THE B4B MLD Project
Discussion.

Coffee

11.15-13.30
B4B AND BIOTECH COLLABORATIONS
Chair Discussants:
S. GELPERINA, R - M. DELI, H

11.15-11.40
P. HASLETT, *SHIRE HGT*
Sanfilippo Syndrome Type A: a study of the natural history of a pediatric neurodegenerative disease
Discussion.

11.50-12.15
A. G. QUINN, *Synageva BioPharma Corp. USA*
Biochemical evidence of the effects of SBC-103, a recombinant human Alpha-n-acetylglucosaminidase in a mucopolysaccharidosis IIIB mouse model using an improved analytical method for substrate quantification
Discussion.

12.25-12.50
E. FENYVESI, *Cyclolab Ltd, Hungary*
Hydroxypropyl-β-Cyclodextrin Excipient becomes an Orphan Drug
Discussion.

13.00-13.25
PFIZER TALK

LUNCH AND FAREWELL
to the next 2014 meeting

NOTES

SEARCHING FOR CHINKS IN THE ARMOR: PERSPECTIVES ON THE NEUROVASCULAR UNIT AND DELIVERY OF CNS THERAPEUTICS

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The treatment of neurological diseases has long been recognized as challenging because of the so-called blood-brain barrier (BBB). The "protective armor" of the brain, consisting of a layer of vascular endothelial cells with low permeability intercellular junctions, was viewed as static and impenetrable, except for a few essential metabolic substrates. During recent years and following major scientific progress a new picture has emerged of a multicellular structure with dynamic form and function called the neurovascular unit (NVU). In addition to endothelial cells, the NVU functions in coordination with pericytes, astrocytes, and neuronal synapses while leucocytes and macrophages also interact physiologically. Rapid signaling among cells of the NVU lead to dynamic responses involving alterations in morphology, transport, cellular trafficking, and communication with other organ systems, thus linking molecular events to physiological consequences.
The endothelial cell layer remains as the principal physical restriction between the serum and brain extracellular space as a consequence of high affinity protein-protein interactions between membrane proteins on adjacent cells. These interactions on the lateral cell surfaces assure very close contact (tight junctions) between cells and restrict diffusion of membrane components, thus enabling asymmetry between the luminal and abluminal membranes. The catalog of facilitated transporters for bringing substances into the brain and existing in either or both the luminal and abluminal membranes is lengthy owing to the many essential substrates, nutrients, cofactors and ions required by the parenchymal cells. By contrast, endothelial cell membranes contain significant numbers of transporters that function to return xenobiotics to the blood, thus utilizing the hydrolysis of ATP to transport substrates against their concentration gradients and preventing their entry into the brain to disrupt neurofunction. Communication with other organ systems via circulating macromolecules and cells is dependent on specific receptors and adhesion molecules located in luminal membrane surfaces. Insulin, transferrin and LRP receptors are well-known examples of many receptors capable of binding their ligand, being endocytosed and trafficking to the abluminal membrane to discharge their respective cargos into the brain extracellular space. Immune cells present in the blood similarly are capable of adherence to endothelial adhesion molecules and through diapedesis migrate into the brain. These events are highly regulated and responsive to numerous and complex signaling pathways.
The need to deliver large molecule therapeutics to the CNS, as represented by lysosomal storage disease treatment, may be accomplished by a number of potential strategies. Currently, these likely are cell-based, receptor- or adsorption-based, nanotechology-based or involve stem cells, viral delivery, transient tight junction disruption, or engage alternative routes such as intranasal delivery. Further research on the fundamentals mechanisms of NVU function is essential to develop the most effective and successful therapeutic strategy.

NEURODEGENERATION AS AN RNA DISORDER

NOEL J BUCKLEY[1] AND RORY JOHNSON[2]

[1] King's College London, Institute of Psychiatry, Department of Neuroscience & Centre for the Cellular Basis of Behaviour & Centre for Neurodegeneration Research, London, UK;

[2] Centre for Genomic Regulation, Bioinformatics and Genomics Group, Barcelona, Spain

Neurodegenerative diseases (NDDs) constitute one of the single most important public health challenges of the coming decades, and yet we presently have only a limited understanding of the underlying genetic, cellular and molecular causes. As a result, no effective disease-modifying therapies are currently available, and no method exists to allow detection at early disease stages, and as a result diagnoses are only made decades after disease pathogenesis, by which time the majority of physical damage has already occurred. Although NDDs are variously classified according to symptomology, natural history, genetics and neuronal loss, there is an emerging consensus that the underlying molecular mechanisms are common to many NDDS. The vast majority of studies have focussed on the roles of specific proteins in mediating neurodegeneration. However, since the sequencing of the human genome, we have come to appreciate that the transcriptional output of the human genome is extremely rich in non-protein coding RNAs (ncRNAs). This heterogeneous class of transcripts is widely expressed in the nervous system, and is likely to play many crucial roles in the development and functioning of this organ. Most exciting, evidence has recently been presented that ncRNAs play central, but hitherto unappreciated roles in neurodegenerative processes. I will review the groundswell of data that supports the view that RNA dysfunction plays a central role in NDDs and in particular, I will focus on the diverse available evidence demonstrating involvement of ncRNAs in NDDS, and discuss their possible implications in the development of therapies and biomarkers for these conditions.

GLUCOCEREBROSIDASE DEFICIENCY AND PARKINSON'S DISEASE

MATTHEW E. GEGG[1], MICHAEL J. DEVINE[2], LAURA OSELLAME[3], MICHAEL R. DUCHEN[3], ANTHONY H.V. SCHAPIRA[1]

[1] Departments of Clinical Neuroscience, [2] Molecular Neuroscience, Institute of Neurology, University College London, UK. [3] Department of Cell and Developmental Biology, University College London, UK.

Mutations in the GBA gene are the greatest known genetic risk factor for developing Parkinson's disease (PD). GBA encodes for the lysosomal hydrolase glucocerebrosidase (GCase) and lysosomal dysfunction has been implicated in PD pathogenesis. A hallmark of PD is the presence of protein inclusions within dopaminergic neurons known as Lewy bodies. The predominant protein in Lewy bodies is alpha-synuclein. This protein is usually turned over by the autophagy-lysosomal pathway. Lysosomes also degrade damaged mitochondria via mitophagy. Perturbed mitochondrial function and mitophagy also occur in PD. In several mammalian cell models, we and others have found that GCase deficiency results in inhibition of autophagy flux and is co-incident with the accumulation of alpha-synuclein and mitochondria.

Post-mortem analysis of PD brains with GBA mutations demonstrated a significant decrease in GCase activity, with deficiency most pronounced in the substantia nigra, the brain region most affected in PD. Interestingly, we also observed GCase deficiency in the substantia nigra of sporadic PD brains, thus confirming the importance of GCase in a large proportion of PD cases. The loss of GCase activity in both groups was associated with a decrease in protein expression. The trapping of mutant GCase in the endoplasmic reticulum (ER), and subsequent ER-associated degradation, may account for the decrease in protein expression in PD brains with GBA mutations. However, we have also found that increased levels of alpha-synuclein in cell culture models result in decreased wild-type GCase protein levels. alpha-synuclein increased the amount of wild-type GCase becoming trapped in the ER.

A bi-directional relationship between GCase and alpha-synuclein appears to occur within the brain. The decreased activity of mutant GCase contributes to the accumulation of alpha-synuclein and mitochondria by inhibiting autophagy. Accumulation of alpha-synuclein can then decrease wild-type GCase thus accelerating and exacerbating PD pathology.

THE ASSOCIATION BETWEEN GAUCHER DISEASE AND PARKINSON DISEASE: FROM HUMAN TO *DROSOPHILA*

GALI MAOR[1], SIGAL RENCUS-LAZAR[1], MIRELLA FILOCAMO[2], HERMANN STELLER[3], DANIEL SEGAL[4] AND MIA HOROWITZ[1]

[1] Department of Cell Research and Immunology, Tel Aviv University, [2] Centro di Diagnostica Genetica e Biochimica delle Malattie Metaboliche, IRCCS G. Gaslini, Genova, Italy, [3] Howard Hughes Medical Institute, Strang Laboratory of Cancer Research, The Rockefeller University, New York, NY 10021, USA, [4] Department of Molecular Biotechnology and Microbiology, Tel Aviv University, 69978, Israel.

Gaucher disease, an autosomal recessive disease, results from mutations in the GBA1 gene, encoding the lysosomal enzyme acid β glucocerebrosidase (GCase). Mutant GCase variants undergo ERAD, the degree of which is a major determinant of disease severity. The presence of mutant molecules in the ER induces ER stress and the unfolded protein response (UPR).

Previous publications noted UPR in GD derived skin fibroblasts. We have extended these studies to show that UPR exists in GD derived skin fibroblasts, manifested by upregulation of the expression of the transcription factor CHOP and the chaperone BiP (Grp78), phosphorylation of eIF2α and cytoplasmic splicing of the transcription factor Xbp1. UPR exits also in skin fibroblasts that derived from carriers of GD mutations. We assume that ERAD of mutant GCase and UPR are a major determinant in the development of Parkinson disease among GD patients and carriers of GD mutations. To confirm this assumption we developed Drosophila models for carriers of GD mutations.

There are two GBA1 homologs in Drosophila, designated CG31414 and CG31148, both encoding proteins showing ~31% identity and ~49% similarity to the human GCase. There are two Drosophila lines available, each with a transposable element insertion (a minos insertion) in one of the fly GCaseorthologs, expected to result in a truncated protein. We tested UPR in double heterozygous flies, as a model for carriers of GD mutations. Our results showed activation of the UPR machinery in the heterozygous flies as tested by Hsc70 (the fly BiPortholog) activation, Xbp1 splicing and phosphorylation of eIF2α. We also established fly lines expressing the human N370S and the L444P mutations. Both lines portrayed UPR and climbing difficulties, reminiscent of Parkinson disease.

To summarize, UPR is a determinant in the development of Parkinson disease among GD patients and carriers of GD mutations.

PROTEOMIC ANALYSIS OF MOUSE MODELS OF NIEMANN-PICK C DISEASE REVEALS ALTERATIONS IN THE STEADY-STATE LEVELS OF LYSOSOMAL PROTEINS WITHIN THE BRAIN

DAVID E. SLEAT[1,2], JENNIFER A. WISEMAN[1], ISTVAN SOHAR[1], MUKARRAM EL –BANNA[1], HAIYAN ZHENG[1], DIRK F. MOORE[3] AND PETER LOBEL[1,2]

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Abstract

Niemann-Pick C disease (NPC) is a neurodegenerative lysosomal disorder characterized by storage of cholesterol and other lipids caused by defects in NPC1, a transmembrane protein involved in cholesterol export from the lysosome, or NPC2, an intralysosomal cholesterol binding protein. Alterations in lysosomal activities have been implicated in NPC pathogenesis therefore the aim of this study was to conduct a proteomic analysis of lysosomal proteins in mice deficient in either NPC1 or NPC2 to identify secondary changes that might be associated with disease. Lysosomal proteins containing the specific mannose 6-phosphate modification were purified from wild-type and Npc1-/-and Npc2-/- mutant mouse brains at different stages of disease progression and identified by bottom-up LC-MS/MS and quantified by spectral counting. Levels of a number of lysosomal proteins were significantly altered, including proteases and glycosidases. In addition, several enzymes involved in lipid catabolism were increased in both forms of NPCin liver and brain, possibly representing a compensatory cellular response to the accumulation of glycosphingolipids. Changes in lysosomal protein levels corresponded with similar alterations in activities and transcript levels. β-hexosaminidase activity altered quite dramatically in response to the loss of either NPC1 or NPC2 in liver and brain, thus we measured total β-hexosaminidase and β-hexosaminidase A activity in serum from control and NPC mutant animals. Both activities were elevated in the serum of the NPC mutants. Understanding the rationale for such changes may provide insights into the pathophysiology of NPC. In more general terms, similar studies in other lysosomal storage diseases may reveal secondary alterations that have value as surrogate markers for disease progression and response to therapy.

INTRA-LYSOSOMAL ACCUMULATION OF FREE $ZN^{2}+$ IN NIEMANN-PICK DISEASE TYPE C1 CORRELATES TO NEURONAL LOSS AND CAN BE TREATED WITH CHELATORS

EMILY CLARK[1], KATHARINA STUMPENHORST[2], HELEN WALLER-EVANS[1], MATHEW WALKER[1], LAURA FRANCIS[1], KAREN FINN[1], FRANCES M. PLATT[2] AND EMYR LLOYD-EVANS[1]

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Niemann-Pick disease type C1 (NPC) is a rare autosomal recessive inherited lysosomal storage disorder affecting children and adults. Patients present with a number of phenotypes including cerebellar ataxia, caused by progressive loss of cerebellar Purkinje neurons, and hallmarks of Alzheimer's disease pathology. At the cellular level the disease is characterized by intra-lysosomal accumulation of multiple lipids including cholesterol, sphingomyelin, glycosphingolipids and sphingosine, endocytic transport defects and lysosomal Ca2+ signaling abnormalities. The disease is caused by mutations in either of two genes, NPC1 or NPC2, which encode a transmembrane protein of the lysosome membrane (NPC1) and a soluble intra-lysosomal protein (NPC2) respectively. Both proteins are believed to be involved in cholesterol transport, although evidence for direct efflux of cholesterol out of lysosomes mediated by either protein is limited.

Having previously characterized a defect in lysosomal Ca2+ signaling in NPC cells, we have now discovered, whilst attempting to identify the protein responsible for the Ca2+ problem, a novel intra-lysosomal accumulation of free Zn2+ in NPC disease. We have determined the presence of this phenotype in all cells measured to date (NPC1 and NPC2 null human patient fibroblasts, NPC1 null CHO, Schwann, astrocytes and macrophages) and it is specific to NPC disease as we did not observe this accumulation in fibroblasts from any other lysosomal disease (Tay-Sachs, GM1 gangliosidosis, mucolipidosis type IV, metachromatic leukodystrophy, Farber, mucopolysaccharidosis type II). In addition to cells in culture, we have discovered elevations in free Zn2+ by histochemistry in the NPC mouse brain using the histochemical free Zn2+ probe TSQ. We found a striking correlation between neuronal loss and the presence of free Zn2+ during the NPC disease process, and interestingly, we found accumulation of Zn2+ in all cerebellar Purkinje cells apart from lobe X, where the neurons appear to be resistant to death.

We will present our recent findings on the potential cause of lysosomal Zn2+ accumulation in NPC, and the cellular pathways that lead to cell death as a result of abnormal Zn2+ levels. Finally, we will present our preliminary data utilizing a screen of heavy metal chelators to reduce intra-lysosomal Zn2+ levels and the effects that they have on NPC cellular function. Our studies highlight a previously unknown NPC phenotype that constitutes a new therapeutic intervention point for the disease.

ROLE OF MEMBRANE CHOLESTEROL IN NEURONAL SURVIVAL AND PLASTICITY IN THE OLD BRAIN

CARLOS G. DOTTI
Centro de Biología Molecular Severo Ochoa, CSIC/UAM, Madrid, Spain

We are trying to understand the mechanisms responsible for the loss of cognitive abilities that accompany aging. In an early work we reported that brain aging is paralleled by the gradual but persistent change in the cholesterol levels in the plasma membrane of hippocampal neurons, both in vitro and in situ (Martin et al., 2008), an area of the brain with implications in learning and memory. We then demonstrated that much of this loss is due to the transcriptional up-regulation of a gene involved in cholesterol hydroxylation: 24 cholesterol hydroxylase, or Cyp46A1, in turn the consequence of metabolic stress from excitatory neurotransmission, therefore the accumulated physiological response to normal brain activity (Sodero et al., 2011, 2012). In functional terms, the change in cholesterol/sphingomyelin content increases the clustering of receptor tyrosine kinase in the plasma membrane of the aging neurons, especially TrkB, helping the survival response of old neurons to exogenous stressors (Martin et al., 2011). On the negative side, cholesterol alterations with age results in impaired lateral mobility and internalization of glutamate receptors of the AMPA type, reducing the capacity of old neurons to efficiently support certain forms of electrical response involved in learning and memory(unpublished). At the molecular level, the lipid imbalance occurring with age leads to the diffusion away from synaptic sites of the cholesterol and PI(4,5)P2 binding molecule MARCKS. Such loss impacts on the amount of PI(4,5)P2 to be hydrolyzed by PLCg and therefore the reduced activation of memory genes(Trovo et al., 2013).

References

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NEURONAL HALLMARKS OF IMPAIRED LYSOSOMAL FLUX: RELEVANCE TO LYSOSOMAL STORAGE DISEASES AND ALZHEIMER'S DISEASE

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The accumulation of cellular waste within organelles of the endosomal-autophagic-lysosomal (EAL) system is a common hallmark found in many neurodegenerative conditions, from rare lysosomal storage diseases to prevalent age-related diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). We have previously shown that autophagic vacuoles (AVs) accumulate in neurons from human AD brains and multiple mouse models of lysosomal storage diseases (Niemann Pick Type C1, Sandhoff disease, GM1 Gangliosidosis), due to impaired catabolic processing by lysosomes (lysosomal flux). Lysosomal enzymes constitutively degrade many potentially neurotoxic substrates, including metabolites of amyloid precursor protein (APP), which contribute to senile plaque formation in AD. In particular, lysosomes cataboliseC-terminal fragments of APP (APP-CTFs) that contain amyloid-β-protein (Aβ), as well as free Aβ itself, and therefore serve an important role as an anti-amyloidogenic/neuroprotective mechanism.
It remains unknown whether impaired lysosomal flux arises in the AD brain due to (i) inefficient lysosomal enzyme activity(primary flux impairment) or (ii) inefficient cargo delivery to lysosomes (secondary flux impairment). It is therefore important to determine if one or both of these mechanisms becomes dysfunctional in AD. By utilising mouse models of lysosomal storage diseases, pharmacological treatments of cultured rat neurons, and post-mortem AD brain tissue, we are currently developing a biomarker profiling system that differentiates primary from secondary lysosomal flux impairment. We have recently identified three lysosomal enzymes (cathepsin B, cathepsin L and tripeptidyl peptidase-1 (TPP-1, CLN2)) that differentially digest APP-CTFs and accumulate "signature CTF profiles" when their activity is inhibited. Interestingly, inactivation of TPP-1 activity caused a disproportionally greater accumulation of amyloidogenic APP-CTFs, which may have implications for the CLN2 form of Batten's disease. Also, by pharmacologically blocking delivery of APP-CTFs to lysosomes, using U18666A (a class II amphiphile and inhibitor of Niemann Pick Type C1 protein) we obtained a fourth APP-CTF profile that is indicative of impaired endosome-lysosome fusion (secondary flux impairment). Current characterization of APP-CTF signature profiles in cerebrospinal fluid, purified B cells and post-mortem brain tissue of people with and without AD and Batten's disease, will determine if different APP-CTF profiles exist and how they relate to our in vitro profiling system. We are also exploring the possibility that an underlying lysosomal dysfunction is apparent in live B cells purified from AD cases, and are developing pharmacological approaches to remediate lysosomal flux impairment. By addressing fundamental reasons why neuron function becomes impaired in lysosomal storage diseases and AD, we aim to improve our understanding of their pathogeneses and identify new diagnostic and therapeutic strategies.

THE PATHOGENESIS OF NEURONOPATHIC GAUCHER DISEASE

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Gaucher disease (GD) is caused by defective activity of the lysosomal enzyme glucocerebrosidase (GlcCerase), which results in accumulation of the glucosylceramide (GlcCer). The rare neuronopathic forms of GD (nGD) are characterized by devastating neurological disease and neuronal loss, but little is known about the processes leading from GlcCer accumulation to neuronal death. We systematically examined the onset and progression of various neuropathological changes in a mouse model of neuronopathic GD, defined the specific brain areas involved (some of which were also reported to be involved in the human disease) and established that localized microglial activation and astrogliosis are spatially and temporally correlated with selective neuron loss (Farfel-Becker et al., 2011). To address the relation between nGD pathogenesis and sphingolipid accumulation, we used mass spectrometry (ESI-MS/MS) and determined that GlcCer levels are rapidly increasing with disease progression, while glucosylsphingosine levels are only moderately elevated and the levels of other sphingolipids, such as galacotosylceramide and ceramide remain unaltered. Finally, to determine the primary neuronal changes upon GlcCer accumulation, we performed microarray analysis on the VPM/VPL of the thalamus, at two stages of the disease, and found a reduction in synaptic genes, ion channels genes and axon guidance genes. We also examined the morphological changes that occur in neurons during degeneration, including misshaping of neuronal soma, neurite loss, and dendritic and axonal beading. Together, our results shed light on the pathogenesis of neuronopathic GD, and may also pave the way for new therapeutic approaches.

CELLULAR AND MOLECULAR PATHOLOGY IN SANFILIPPO SYNDROME

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Progressive mental deterioration in Sanfilippo syndrome (MPSIII) presumably results from combined deleterious processes affecting the CNS, which are turned on by heparan sulfate oligosaccharide accumulation. According to clinical manifestations in children, it is presumable that damages primarily concern events occurring during cortical maturation. Identification of pathogenic mechanisms at the cellular level is necessary to anticipate benefit of treatments and will impact on patient eligibility criteria and endpoints of future clinical trials.

We generated several models for the study of cellular disorders in MPSIII, which provided consistent and complementary new insights in the molecular pathology of this disease.

We showed in MPSIIIB mouse neurons that intracellular vacuoles are not related to the endocytic or macro-autophagy pathways [1]. These results were not consistent with the common view, which favours the hypothesis that vacuoles are formed as the consequences of reactive proliferation and swelling of lysosomes in response to lysosomal function deficiency. In contrast, we produced evidence for alterations of Golgi structure and Golgi matrix protein GM130 functions. GM130 is a multifunctional protein involved in the control of Golgi size and organisation with GRASP65, microtubule nucleation at Golgi membranes with AKAP450, cell polarity with YSK1/stk25, and centrosome organisation with the Cdc42 guanine exchange factor TUBA.

In addition to MPSIIIB mouse neurons, we observed alteration of Golgi structure and expanded expression of GM130 in undifferentiated iPSc derived from patient skin fibroblasts and in differentiated neurons derived from these iPS cells [2]. Investigations of these cells provided evidence for alteration of FGF2 signalling, abnormal expression of genes involved in extra-cellular matrix turnover and proteins involved in axonal guidance and remodelling.

Studies in HeLa cells in which shRNA directed against NAGLU are expressed in the presence of tetracycline led to similar conclusions [3]. We demonstrated that Golgi alterations and vacuole formation were caused by GM130 over-expression and associated with increased nucleation of Golgi associated acetylated microtubules by AKAP450.

Our studies in MPSIIIB mouse astrocytes, mouse and human neural stem cells and neurons provided evidence for the activation of FAK by extracellular heparan sulfate fragments [4]. FAK is a tyrosine kinase involved in focal adhesion, cell polarity, cell migration, and axonal remodelling. Constitutive

FAK activation was associated with enhanced focal adhesion, defective polarization, abnormal migration in MPSIII astrocytes and neural progenitors [4], and enhanced neurite outgrowth in MPSIII neurons [5].
According to the results summarized above, we make the hypothesis that the development of neuropathology in MPSIIIB is triggered by HS oligosaccharides bound to the ECM, which modifies cell perception of environmental cues and affects cell migration, neurite development and plasticity during cortical maturation.

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TRANSPORT OF LYSOSOMAL ENZYMES AND HIV-1 FROM BLOOD TO BRAIN BY THE M6P RECEPTOR: EFFECTS OF NEUROINFLAMMATION AND ADRENERGIC REGULATION

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The central nervous system pathology of neonates deficient in lysosomal enzymes can be corrected by the peripheral administration of those enzymes. This raises the question of how those enzymes negotiate the blood-brain barrier (BBB). We found that phosphorylated beta-glucuronidase (P-GUS) and sulfamindase readily crossed the neonatal BBB; in contrast, we found, as have many others, that the neonatal BBB was no more permeable to albumin than the adult BBB. Instead of leaking across the BBB, the enzymes were transported across it by the mannose 6-phosphate receptor (M6P-R), with M6P-R-mediated transport activity greatly abating with aging, so that enzyme was no longer transported across the BBB of adults. We have also found that enzyme transport could be rapidly re-induced by treating mice with epinephrine. Although epinephrine at high doses can disrupt the BBB, we found that transport induction required a dose below that which consistently disrupts the BBB and that enzyme was crossing an intact, adult BBB. We demonstrated by competitive inhibition studies that epinephrine was working by re-inducing transport that was dependent on M6P-R. This suggests that the MP6 receptor is present within the adult BBB, but in an inactivated state that can be activated with adrenergics. Parallel work investigating how HIV-1 free virus crosses the adult BBB found that it can use a M6P receptor to enter the CNS. We had found that HIV-1 transport across the BBB was mediated by glycoprotein interactions and accelerated by activation of the innate immune system. Treatment of in vitro BBB models with lipopolysaccharide, a derivative of the cell wall of gram negative bacteria that activates the innate immune system, induces release of the cytokines IL-6 and GM-CSF from brain endothelial cells; these cytokines then act in paracrine fashion on those brain endothelial cells to induce HIV-1 uptake and transcytosis through a P38 MAPK-dependent pathway. Free HIV-1 uptake is also stimulated by wheatgerm agglutinin and protamine sulfate and work by others has implicated mannose as important to HIV-1 uptake. We found that baseline and stimulated (by WGA or protamine) HIV-1 transport across the in vitro BBB was blocked by mannan, mannose, and mannose 6-phosphate. Treating HIV-1 with endoglycosidase F1, a cleaver of high mannose oligosaccharides, decreased the ability of HIV-1 to cross the BBB. We conclude from this that HIV-1 can co-opted one of the M6P receptors, using it to cross the BBB.

INTERACTION OF ENGINEERED NANOPARTICLES WITH INTERNAL BIOLOGICAL BARRIERS

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Current evidence indicates that engineered nanoparticles (ENPS) may both cross and damage organs protected by internal barriers, possibly at doses and exposure routes expected in occupational and consumer setting. The in vivo studies provide important data on some ENPs showing a relatively high damaging potential, but information on a more extensive panel of consumer–relevant and worker-relevant ENPs is clearly needed. The process of barrier crossing follows probably different dynamics in different barriers, and appropriate long-terme valuations are also needed in order to understand the real hazard posed by exposure to a given ENP. Special consideration should be given to the blood-brain barrier, because of the possible pathophysiologic role of ENP exposure in the development of neurodegenerative disorders. In vivo studies shows that gold nanoparticles, cerium oxide nanoparticles, silver nanoparticles, iron oxide nanoparticles, titanium oxide nanoparticles, and single wall carbon nanotubes, are able to cross the blood-brain barrier after oral or lung exposure, and brain histopathological damage has been demonstrated for some of them. In this perspective, it is very relevant the in utero exposure, since crossing of placental barrier has been reported for several ENPs: Once ENPs gain access into the embryo's body, the brain is a preferential site of accumulation, given the incomplete development of the blood-brain barrier in the very early stages of life. Apoptosis and damage of neural embryo cells was associated with in utero exposure to titanium dioxide nanoparticles and fullerenes, and memory impairment in the offspring of mice exposed to carbon black. We will present data from our ongoing experiments showing the biodistribution of pegylated single wall carbon nanotubes and silica nanoparticles in pregnant mice. It deserves to be pointed out that although the propensity of nanoparticles to cross biological barriers may give rise to unexpected, adverse effects on human health, this could also be exploited for therapeutic gain. Once again, the crossing of the blood-brain barrier is crucial in this respect. We are developing prototypes of low density lipoprotein(LDL)-like ENPs which can be used as nanovectors of diagnostic and therapeutic agents in several disorders, ranging from atherosclerosis, to cancer, to neurodegenerative diseases. The rationale for the use of LDL-like nanoparticles in the treatment of neurological disorders stems from the possible pathogenetic role played by LDL in the development of Alzheimer's disease: in the case of dysfunction or inflammation of the blood-brain barrier, LDL/OxLDL may translocate into the brain, where they may interact with APP to produce Ab Amyloid while at the same time free LDL binds to LRP receptor, which is switched-off, there by impairing Ab Amyloid clearance. This effect may be particularly pronounced in people with the ApoE4 variant, showing the combination of high levels of LDL in the blood, propensity to atherosclerosis (with increased levels of OxLDL) and propensity to Alzheimer's disease. On this basis, LDL-like ENPs might follow the same pathways of native LDL, in order to reach the brain and perform targeted delivery of anti-inflammatory drugs in the early stages of the disorder. We will describe three LDL-like prototypes we are developing for this purpose.

OPENING INTERCELLULAR JUNCTIONS OF BRAIN ENDOTHELIAL CELLS WITH PEPTIDES

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The epithelial and endothelial barriers of the human body are major obstacles for drug delivery to the systemic circulation and to organs with unique homeostasis, like the central nervous system. The intercellular tight and adherens junctions restrict the free passage of hydrophilic or large molecules through the paracellular clefts. Six peptides acting on claudin-4, occludin, E-cadherin, and ZO integral membrane and linker junctional proteins [1] were selected and tested on primary rat brain endothelial cells in co-culture with pericytes and astrocytes, a model of the blood-brain barrier (BBB), and vinblastin-selected human Caco-2 epithelial cells [2], a model of the intestinal barrier. The peptides were checked for cellular toxicity and non-toxic concentrations were used to test their effects on barrier integrity. The peptides decreased the electrical resistance and increased the permeability of brain endothelial cells for marker molecules fluorescein and albumin, except the C-CPE peptide which acts on claudin-4 not expressed at the BBB. All peptides increased the permeability in Caco-2 cells, which was further enhanced in calcium and magnesium ion free conditions. The effect of peptides on intercellular junctions were verified by immunostaining for claudins, ZO-1 and β-catenin. Changes in cell shape were monitored by phase holographic imaging. Peptides FDFWITP and PN-159 were the most effective modulators of junctional permeability in both models. Our results indicate that peptides have a potential to be used as pharmaceutical excipients to improve drug delivery across biological barriers.

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RESEARCH ON RARE DISEASES: INTERNATIONAL AND PATIENT INCLUSIVE

MONICA ENSINI
European Organisation for Rare Diseases - EURORDIS

The European Organisation for Rare Diseases - EURORDIS - is a non-governmental patient-driven alliance of patient organisations and individuals active in the field of rare diseases, dedicated to improving the quality of life of all people living with rare diseases in Europe and beyond. EURORDIS was founded in 1997 and as of February 2013 represents over 550 rare disease patient organisations in more than 50 countries, covering more than 4000 rare diseases. Also, EURORDIS supports the creation and development of rare disease national alliances and disease-specific European federations and networks. Fundamental and clinical research represent for EURORDIS major fields of advocacy actions aimed at influencing public decision-makers to take stance for their robust support on the eve of the adoption of the 8th EU Research Framework Programme 2014–2020 (Horizon 2020). Fostering research on rare diseases in Europe is an effort that must be carried out jointly at the national, European and International level. Indeed in recent years, research on rare diseases has been boosted thanks to the European Commission Framework Programme for Research and Technological Development, a number of different national initiatives adopted across European countries and international programs. One paradigmatic initiative is the International Rare Diseases Research Consortium (IRDiRC) that was launched in April 2011 to foster international collaboration in rare diseases research. IRDiRC will team up researchers and organisations investing on research on rare diseases in order to achieve two main objectives, namely to deliver 200 new therapies for rare diseases and means to diagnose most rare diseases by the year 2020. Patients' involvement and participation to this ambitious program is assured by EURORDIS with its active role in the Consortium management. One of the major projects financed by the EU Commission under the IRDiRC umbrella is RD-Connect. RD-Connect is a unique global infrastructure project that links up databases, registries, biobanks and clinical bioinformatics data used in research on rare diseases into a central resource for researchers worldwide. With a six-year funding provided by the EU but uniting researchers across the world, it will develop an integrated research platform in which complete clinical profiles will be combined and integrated with genomics, transcriptomics, proteomics and metabolomics and detailed phenotype (phenomics) data across centres and across diseases. The achievements of the RD-Connect objectives will represent a major step forward to the delivery of concrete benefits to patients in terms of diagnosis and therapy development. Patients are indeed kept at the core of the project being EURORDIS a full partner that will assure a fruitful partnership between patients, researchers and clinicians in speeding up knowledge production aimed at therapy development, initially focusing on two groups of rare diseases, rare kidney diseases and rare neurodegenerative and neuromuscular diseases (EURenOmics and NeurOmics projects respectively). One of EURORDIS' longstanding guiding principles is the empowerment of patients in order to obtain their recognition as full and equal partners in research on rare diseases, research that they often directly fund and strongly support. Especially in the rare disease field the tight interaction between patients, researchers and clinicians represents a virtuous circle of knowledge production that can be extremely inspiring and beneficial for all participants to this exercise.

INHERITED NEUROMETABOLIC DISEASES INFORMATION NETWORK (INNERMED-INETWORK)

MAURIZIO SCARPA
Representative of the Brains for Brain Foundation, Main Partner Organization
Brains for Brain Foundation c/o Dept. of Pediatrics University of Padova Italy

The Directorate-General for Health & Consumers (DG SANCO) has approved the project "Inherited NeuRoMetabolic Diseases INFORMATION NETWORK" (InNerMeD-I-network) of which the Brains for Brain Foundation is the main leader partner.
NeuroMetabolic Disorders (NMDs) are a group of rare paediatric genetic metabolic disorders that impact on the brain causing mental retardation and progressive neurodegeneration that, if not promptly treated, ends in early death. Lack of awareness of these conditions can lead to delayed diagnosis and start of treatment, with consequent tragic results. Increasing awareness is therefore the first crucial step in fighting these conditions.
InNerMeD-I-network wants to create a network of information targeted on diagnosis and treatment of NMDs based on the collection and exchange of proper information among scientific community, health professionals, patients, patients association and all interested stakeholder. The project aims to increase current knowledge on NMDs and speed up the timely and precise identification of patients to which apply the available treatments. The network will also favor biomedical research, straightening research capacities and fostering innovative therapeutic tools derived from the recent scientific advancements based on biomarkers use and personalised approaches.
Brains for Brain Foundation, coordinator and main leader partner, will particularly assure the partners coherence and capacity to share decisions and to put together relevant knowledge and research capacity.
The partnership also includes both public and private international clinical centres:
Gianni Benzi Foundation (FGB) - (Italy)
Region Hovedstaden (RH) - (Denmark)
Children's Hospital, University of Mainz (UMC-M) - (Germany)
Sveučilište u Zagrebu Medicinski fakultet (UZSM) - (Croatia)
Hospital Sant Joan de Déu (HSJD) - (Spain)
Thanks to partners specific expertise InNerMeD-I networking will create a formidable concentration of competences in such a complex, heterogeneous and niche therapeutic area.

SANFILIPPO SYNDROME TYPE A: A STUDY OF THE NATURAL HISTORY OF A PEDIATRIC NEURODEGENERATIVE DISEASE

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Background: MPS IIIA (Sanfilippo Syndrome type A) is a lysososomal storage disease affecting children that causes accumulation of the glycosaminoglycan, heparan sulfate, and results in progressive neurodegeneration. Understanding of the clinical spectrum of MPSIIIA disease, its rate of progression and how best to measure this, is essential for the efficient development of therapy.
Methods: Twenty four children with MPSIIIA disease were enrolled at a single center and followed for 12 months. Clinical neurodevelopmental assessments using standardized instruments and brain magnetic resonance imaging were performed at baseline, 6 months and 12 months. Cerebrospinal fluid (CSF) was collected for biomarker analysis.
Findings: Children fell into two broad phenotypic groups, distinguished by age of diagnosis. Nineteen patients diagnosed under the age of 6 years exhibited rapid declines in neurodevelopmental status, expressed as a developmental quotient (DQ), over 12 months of observation. All but one of these patients with "classical" disease showed no development past a cognitive age of 28 months. The remaining children were diagnosed after age 6, and showed variable rates of disease progression. Automated volumetric analysis of brain magnetic resonance images revealed consistent declines over 12 months in cortical grey matter volume among children with classical disease, which correlated strongly with declines in DQ. Preliminary CSF analysis revealed elevation of heparan sulfate levels which varied between patients, but appeared relatively stable within patients over 6 or 12 months of observation.
Conclusion: Classical MPSIIIA disease is characterized by consistent declines in neurodevelopmental status readily measurable over 12 months, which correlate with declines in objectively measured cortical grey matter volume. These findings suggest the applicability of these measures in clinical trials of therapy. The early age at which a cognitive developmental ceiling is reached suggests the need for early therapeutic intervention to obtain maximum benefit.

BIOCHEMICAL EVIDENCE OF THE EFFECTS OF SBC-103, A RECOMBINANT HUMAN ALPHA-N-ACETYLGLUCOSAMINIDASE IN A MUCOPOLYSACCHARIDOSIS IIIB MOUSE MODEL USING AN IMPROVED ANALYTICAL METHOD FOR SUBSTRATE QUANTIFICATION

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Mucopolysaccharidosis IIIB (Sanfilippo B Syndrome) is a lysosomal storage disorder caused by deficiency in the enzyme alpha-N-acetyl-glucosaminidase (NAGLU). At present there is no treatment for this disease. We have previously described methodologies for the production of SBC-103, a recombinant human NAGLU with enhanced mannose-6-phosphate-dependent cellular uptake which is in marked contrast to earlier unsuccessful attempts to produce rhNAGLU with good uptake properties using cell culture methods (Weber B et al, 2001).

Given the limitations of historical methods used for NAGLU substrate analysis, which show minimal discrimination in brain GAG content between normal and NAGLU deficient mice, we have developed an analytical method based on SAX-HPLC analysis of heparan sulfate disaccharides (HSD). Using this method we have demonstrated a > 4-fold increase in brain HSD content by 3 months of age in NAGLU deficient mice. Liver and kidney tissue demonstrated a > 25-fold increase.

In order to determine the sensitivity of the HSD method for detecting improvements in response to enzyme delivery, we have also analyzed tissue HSD levels from the brain and liver of wild type, treated and untreated NAGLU deficient mice. In pilot studies with different dosing approaches, we have established that increases in tissue enzyme activity are accompanied by dose dependent reductions in HSD levels.

The ability to quantify accumulated substrate in the MPS IIIB disease model and our pilot data on the effects of enzyme replacement on tissue HSD levels in this model supports further investigation of SBC-103 as a therapy for patients with MPS IIIB.

HYDROXYPROPYL-B-CYCLODEXTRIN EXCIPIENT BECOMES AN ORPHAN DRUG

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2-hydroxypropyl-beta-cyclodextrin (HPBCD) has long been used as a potent solubilizer for water-insoluble drugs [1]. The well-tolerated solubilizer excipient is approved in a number of parenteral pharmaceutical dosage forms (e.g. Sporanox®, Dyloject®, etc)
This cyclodextrin derivative is a composite, isomeric mixture, X-ray amorphous, non-crystallizable, highly soluble in water. The solubilization power is based on its inclusion complex forming potency, a non-covalent interaction between drug and sub-nanometer-sized HPBCD cavity.
HPBCD "enjoys" broad acceptance criteria in the US and European Pharmacopoeia in terms of degree of substitution (DS). The acceptance range is between DS =2.8 and DS =10.5. Both the degree and the pattern of substitution affect the physico-chemical properties, solubilizing potency, aggregation and other functional properties of the HPBCD products.
The presentation attempts to evaluate in detail the role of DS and substitution pattern on the functional properties (solubilization effect, solution clarity/precipitation, cholesterol mobilization, aggregation tendency etc.) of HPBCDs of different origin with low, medium and high DS, all satisfying pharmacopoeia requirements. Tailored analytical methods (HPLC/MS/MS, MALDI, 13C 1HNMR) will illustrate together with cholesterol solubilization results, the remarkable cholesterol and other lipid solubilizing power of different commercial HPBCDs. This presentation also demonstrates the performance difference between different HPBCDs having different DS. Strengths and weaknesses of the applied Pharmacopoeia and other analytical methods will be discussed in the light of DS determination errors that often occur.
CycloLab's recommended analysis methods for HPBCD qualifications are now being introduced by EMEA European Agency and will soon be official.
Based upon the presented analytical and complexation/solubilization results, the most suitable types of HPBCDs for in vivo cholesterol mobilization and for therapeutic uses will be proposed [2]. This parenterally safe cholesterol-complexing solubilizer recently received Orphan Drug designation for the treatment of Nieman Pick type C disease.

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NOTES

THIS
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ACTELION

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