



European Working Group on Gaucher Disease



9th International EWGGD Workshop



Program and Abstracts!

June 30th – July 3rd 2010

Cologne | Germany



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European Working Group on Gaucher Disease

9th International EWGGD Meeting

Organizing Group



Bembi, Bruno



Hollak, Carla



Hrebiczek, Martin



Manuel, Jeremy



Niemeyer, Pascal



vom Dahl, Stephan

Dear colleagues, physicians and scientist, dear visitors

it is our pleasure to welcome you to the 9th Workshop of the EWGGD (European Working Group on Gaucher Disease). This time, the location will be Grand Hotel Schloss Bensberg near Cologne, in Germany. Hopefully, the calm atmosphere and the famous view on one of Germany's oldest cities, Cologne, will add to your well-being and stimulate scientific ideas.

As in the former years, the principal aim of the meeting is to enable a fruitful scientific exchange on Gaucher-related issues. Young physicians and researchers from all scientific backgrounds are encouraged to present their research and to attend our meeting for learning purposes. The opportunity for presenting unpublished scientific data as well as free discussion is a central premise of the Group.

A couple of things were novel this time: The European Gaucher Alliance (EGA), the head organisation of patient associations in Europe, was involved into the organisational flow of the workshop from the beginning. Second, a travel grant programme has been set up to support the attendance of young researchers and physicians to present their results. During this meeting, the posters will not only be displayed, but discussed during separate poster tours on Thursday and Friday. Fourth, this time we will try to shape an organisational frame for the EWGGD, a constitution, in order to become a fully appreciated daughter of ESGLD (European Study Group on Lysosomal Diseases).

We also would like to thank the sponsors, who support this meeting generously. Without their support, an ambitious international meeting would hardly be possible.

During this meeting, we will also take the chance to appreciate the work of our long-standing chairmen, Prof. Hans Aerts from Amsterdam and his vice, Prof. Timothy Cox from Cambridge. They have inspired Gaucher work from the beginning on and will hopefully go on with this.

Besides the academic challenge, you may find some rest and relaxation by enjoying the neighbourhood or just watch one of the FIFA 2010 Soccer World Cup quarterfinals. Of course, we will take the opportunity to go out to a local Cologne brewery.

The organization committee welcomes you to EWGGD 2010 and hope you profit intellectually and personally from this workshop.

Stephan vom Dahl, on behalf of the Organizing Committee

Carla Hollak
Martin Hrebiczek
Bruno Bembi
Pascal Niemeyer
Jeremy Manuel

9th Workshop of the EWGGD

(European Working Group on Gaucher Disease)

June 30 – July 3, 2010

Grandhotel Schloss Bensberg

(near Cologne, Germany)

FINAL PROGRAM

as of 28 June 2010

Wednesday, June 30, 2010

Arrival of attendants, reception desk at

Grand Hotel Schloss Bensberg
Kadettenstrasse, D-51429 Bergisch Gladbach
(near Cologne, Germany)
Phone: +49-2204-42969

will be open in the afternoon and evening

please note:

19.30-22.00

Welcome reception with fingerfood drinks

Lobby of Grand Hotel Schloss Bensberg

Thursday, July 1, 2010

Start of lectures: 9:30 am, Ballroom Schloss Bensberg

9.30-9.35 Welcome remarks
S. vom Dahl, Cologne

9.35-10.10 Opening lecture: "From the beginning"
H. Aerts, Amsterdam

Session I Basic research

Chairs: H. Michelakakis, Athens
Martin Hrebicek, Prague

10.10-10.35 Lysosomal integral membrane protein type-2 sorting
receptor of β -glucocerebrosidase: a cell-type specific
mechanism
A. Balreira, Porto

10.35-10.50 Glucocerebrosidase alternative promoter has features and
expression characteristic of housekeeping genes
E. Svobodova, Prague

10.50-11.15 Efficient CNS, PNS and visceral gene delivery following fetal
and neonatal intravenous administration of AAV9 – an
approach for studying type II neuronopathic Gaucher
disease
A. Rahim, London

11.15-11.45 Coffee break

Session II Clinical aspects

Chairs: P. Deegan, Cambridge
A. Tytki-Szymanska

11.45-12.10 Invited lecture: "The potential role of EU politicians in
improving patient outcome and research in orphan diseases"

P. Liese, MEP, Brussels

12.10-12.15 Nomination of Dr. Liese as EGA honorary chairman
J. Manuel, London

12.15-12.35 Influence of c.(-203)>G variant in the glucocerebrosidase
gene in type 1 Gaucher Disease phenotype
P. Alfonso, Zaragoza

- 12.35-12.55 Diffusion Tensor Imaging: Study of brain white matter in paediatric Gaucher Type I and Type III
E. Davies, London
- 12.55-13.15 Osteonecrosis of the head of femur in Gaucher disease: a descriptive study of histological changes
E. Lebel, Jerusalem
- 13.15-14.00 Lunch**
- 14.00-14.45 Poster tour
(Guides: A. Zimran, Jerusalem and H. Michelakakis, Athens), poster presenters 1-20 have to be at their posters, posters will be discussed in two separate groups 1-10, and 11-20 synchronously

Session III Clinical goals

Chairs: N. Belmatoug, Paris
E. Lukina, Moscow

- 14.45-15.00 Helping to optimise patient care in Gaucher Disease: A novel assessment and monitoring tool for therapeutic Goals
G. Pastores, New York, NY
- 15.00-15.15 Longterm Outcome with ERT in 26 German pediatric Gaucher patients
A. Heidrich, Mainz
- 15.15-15.30 Bone mineral density responses to enzyme replacement therapy in pediatric patients
G. Ciana, Udine
- 15.30-15.45 Significant and continuous improvement in bone mineral density among type 1 Gaucher disease patients treated with velaglucerase alfa: 69-month experience, including dose reduction
D. Elstein, Jerusalem

15.45-16.15 Coffee break

Session IV Shortage of imiglucerase

Chairs: T. Cox, Cambridge
M. Beck, Mainz

Status of current situation in

16.15-16.30	Israel and Australia	A. Zimran, Jerusalem
16.30-16.35	The Netherlands	L. v. Dussen, Amsterdam
16.35-16.40	Spain	P. Giraldo, Zaragoza
16.40-16.45	Italy	A. Saccari, Udine
16.45-16.50	France (on behalf of CETG (Comité d'Evaluation du Traitement de la maladie de Gaucher), Paris	N. Belmatoug
16.50-16.55	United Kingdom	D. Hughes, London
16.55-17.00	Cerezyme Emergency Treatment Program	C. Hollak, Amsterdam
17.00-17.15	Discussion	All

The companies' standpoints

17.15-17.30	Genzyme	O. Amitay, Boston, MA
17.30-17.35	Protalix Biotherapeutics	D. Aviezer, Carmiel
17.35-17.40	Actelion	O. Morand, Basel
17.40-17.45	Shire HGT	M. Rothera, Boston, MA
17.45-18.15	Discussion	All

19.30 Departure with tram No. 1 to Köln citycentre, social event in local brewery (please note: 10 min walk from the hotel to the tram, will you still be able to make it?)

Location: Kölner Hofbräu, P-Josef-Früh KG, Am Hof 12-18,
50667 Köln (opposite to the Cathedral), Tel. +49-221-26130

23.00 Return with buses from Köln to hotels

Friday, July 2, 2010

Session V Patient perspectives and registries

Chairs: Carla Hollak, Amsterdam
Jeremy Manuel, London

- 8.45-9.00 Choices and challenges
T. Collin-Histed, Gloucestershire; P. Niemeyer, Luxembourg
- 9.00-9.15 Registries: benefits and limitations
C. Hollak, Amsterdam
- 9.15-9.25 Genzyme: ICGG
R. Moscicki, Boston, MA
- 9.25-9.30 Shire Human Genetic Therapies: GOS
A. Conway, Boston, MA
- 9.30-9.35 Actelion: IS3
O. Morand, Basel
- 9.35-9.40 Pfizer/Protalix Biotherapeutics
R. Urbanski, New York, NY
- 9.40-10.00 Discussion
All

10.00-10.30 Coffee Break

Session VI Neurological aspects

Chairs: H. Rosenbaum, Haifa
H. Aerts, Amsterdam

- 10.30-10.55 Invited lecture: "Parkinsonism and Gaucher disease"
E. Sidransky, Bethesda, MD
- 10.55-11.15 Early signs of Parkinson's disease in Gaucher patients
T. Boettcher, Rostock
- 11.15-11.30 Four year follow up of Type III Gaucher patients using a modified Severity Scoring Tool
E. Davies, London
- 11.30-11.50 Type 1 Gaucher disease patients exhibit cognitive function deficits: results of a two-year prospective observational study
M. Biegstraaten, Amsterdam

11.50-12.05 Neuronopathic Gaucher disease: Follow Up and Longterm Outcome in 30 German Patients
E. Mengel, Mainz

12.05-13.00 Lunch

13.00-14.00 Poster tour II (Guides: S. vom Dahl, Cologne and M. Hrebiczek, Prague), poster presenters 21-45 have to be at their posters, posters will be presented in 2 groups (21-32, and 33-45 synchronously)

Session VII Pathophysiology

Chairs: P. Giraldo, Zaragoza
M. Horowitz, Tel Aviv

14.00-14.25 Parkin-mediated ubiquitination and degradation of mutant glucocerebrosidase variants-a possible link between GD and Parkinson disease
M. Horowitz, Tel Aviv

14.25-14.50 The link between innate immunity and Gaucher disease
O. Goker-Alpan, Springfield, VA

14.50-15.10 Reducing glycosphingolipids restores insulin sensitivity in obese mice
M. van Eijk, Amsterdam

15.10-15.30 Role of GBA2 in Gaucher disease
Y. Yildiz, Bonn

15.30-16.00 GBA1-deficient mice recapitulates Gaucher's disease displaying system-wide cellular and molecular dysregulation beyond the macrophage: evidence for an osteoblastic bone formation defect underlying osteopenia
P. Mistry, New Haven, CT

16.00-17.00 Coffee Break (with football viewing option)

17.00-19.00 **Business Meeting of the EWGGD** (to be postponed by 45 min, if Soccer World Cup 1st quarterfinal of outstanding interest, match should end by 17.45)

Chairs: S. vom Dahl, Cologne
B. Bembi, Udine

EWGGD Business meeting in Amsterdam 2009 (C. Hollak)
Discussion on potential EWGGD constitution (J. Manuel)
Election of new EWGGD board members (S. vom Dahl)
Election of organizer of 10th workshop 2012 (S. vom Dahl)

19.30-22.30 Buffet dinner at the Grand Hotel Schloss Bensberg (including opportunity to see 2nd quarterfinal)

After buffet dinner, transfer to hotels outside Grand Hotel will be organized

Saturday, July 3, 2010

Session VIII New aspects and novel therapies

Chairs: P. Mistry, Yale, CT
A. Mehta, London

9.00-9.25 Imiglucerase biosimilar development for Gaucher disease
H. Park, Seoul

9.25-9.50 Novel enzyme replacement therapy for Gaucher disease: phase III pivotal clinical trial with plant cell expressed recombinant glucocerebrosidase (prGCD) - taliglucerase alfa
A. Zimran, Jerusalem

9.50-10.10 Enzyme replacement therapy with velaglucerase alfa significantly improves key clinical parameters in type I disease: positive results from a randomized double-blind, global phase III study
A. Zimran, Jerusalem

10.10-10.30 Whole body MRI in type I Gaucher patients: preliminary results of bone involvement
L. Poll, Duisburg

10.30-11.00 Coffee break

Chairs: V. Gieselmann, Bonn
G. Pastores, New York, NY

11.00-11.10 Antigenic differences in patients with type 1 Gaucher disease receiving velaglucerase alfa or imiglucerase enzyme replacement therapy in controlled clinical trials
J. Lyczak, Cambridge, MA

11.10-11.20 Safety and efficacy of velaglucerase alfa in Gaucher disease type 1 patients previously treated with imiglucerase
P. Giraldo, Zaragoza

11.20-11.40	A novel, ultra-sensitive technique to visualize active glucocerebrosidase W. Kallemeijn, Amsterdam
11.40-11.55	Eliglustat tartrate, An investigational oral compound for Gaucher disease type 1 (GD1): phase 2 results after 2 years E. Lukina, Moscow
11.55-12.15	SP2-Iminisugars as pharmacological chaperones for Gaucher disease: mutation profiling, cellular uptake and intracellular distribution studies Z. Luan, Yonago
12.15	An appreciation of the work of Prof. Bruno Berra, Italy Prizes and thanks B. Bembi, Udine
12.15	Invitation to the next EWGGD meeting organizer next meeting
12.25	Closing remarks: "To the future" S. vom Dahl, Cologne
12.30	End of meeting
12.30-13.00	Small lunch before departure Departure to trains, cars and airports

Thank you for being with us, have all a safe journey back home.

LYSOSOMAL INTEGRAL MEMBRANE PROTEIN TYPE-2 SORTING RECEPTOR OF β -GLUCOCEREBROSIDASE: A CELL-TYPE SPECIFIC MECHANISM

Balreira Andrea¹, Gaspar Paulo¹, Caiola Daniel¹, Chaves João⁴, Beirão Idalina⁵, Lima José Lopes⁴, Macário Maria do Carmo⁶, Matos Anabela⁶, Azevedo Jorge Eduardo^{2,3} and Sá Miranda Maria Clara¹

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email:mcsamir@ibmc.up.pt

Objectives

Alterations in *SCARB2* gene that codes for Lysosomal Integral Membrane Protein type 2 (LIMP-2), were recently described in patients with Action Myoclonus Renal Failure Syndrome (AMRF) (1,2). AMRF is an autosomal recessive progressive myoclonic epilepsy without intellectual impairment associated with renal failure.

Here we present the characterization at the clinical, biochemical and molecular levels of Portuguese patients with AMRF from two apparently unrelated consanguineous families. We aim to call the attention to this rare disease trying to contribute for the identification of undiagnosed patients.

Methods and results

Leukocytes and cultured skin fibroblasts from patients and their parents were used to determine β -glucocerebrosidase activity, to characterize LIMP-2 at the protein level and to analyze the *SCARB2* and *GBA* genes.

The sequence analysis of the exonic regions and intronic boundaries of *GBA* gene failed to reveal the existence of mutations in the gene encoding β -glucocerebrosidase. In contrast, the sequencing of the gene encoding LIMP-2 revealed a non-sense mutation in codon 178 (W178X) in homozygosity. The possibility of a common ancestor in both families is being investigated.

Molecular studies carried out in cultured skin fibroblasts of patients showed the absence of immunodetectable LIMP-2, decreased amounts of β -glucocerebrosidase with an abnormal glycosylation pattern and mainly located in the endoplasmic reticulum, as assessed by its sensitivity to Endo H.

The results showed, for the first time, that the patients with AMR present a severe β -glucocerebrosidase deficiency in cultured skin fibroblasts but a normal enzyme activity in leukocytes.

Conclusion

Our results showed, for the first time, that the patients with AMR present a severe β -glucocerebrosidase deficiency in cultured skin fibroblasts but a normal enzyme activity in leukocytes. Accordingly, the gene encoding LIMP-2 must be analyzed in all individuals presenting a cell-type deficiency of β -glucocerebrosidase.

1 Berkovic SF et al, Am J Hum Genet. 2008 Mar;82(3):673-84.

2 Balreira A et al, Hum Mol Genet. 2008 Jul 15;17(14):2238-43.

GLUCOCEREBROSIDASE ALTERNATIVE PROMOTER HAS FEATURES AND EXPRESSION CHARACTERISTIC OF HOUSEKEEPING GENES

Eva Svobodová¹, Lenka Mrázová¹, Ondřej Lukšan², Larisa Stolnaja¹, Lenka Dvořáková¹, Milan Jirsa², Martin Hřebíček¹

¹*Institute of Inherited Metabolic Disorders, Charles University, 1st Faculty of Medicine and University Hospital, Prague*

²*Laboratory of Experimental Hepatology, Institute of Clinical and Experimental Medicine, Prague*

Glucocerebrosidase transcripts show alternative splicing at their 5' ends, suggesting that a fraction of the transcripts originates at an alternative upstream promoter (P2) located 2.6 kb upstream of the first ATG. Currently, there are five alternative GBA transcripts in the databases, one of which is transcribed from the downstream (P1) promoter and four others apparently from the P2 promoter. The alternative transcripts from the putative upstream promoter contain one or two extra exons (exon -2 or exons -2, -1, respectively), but the first ATG codon and predicted amino-acid sequence are the same as in the transcript from the downstream P1 promoter. Our goal was to confirm that the putative P2 functions as a promoter and to study its properties.

The in-vitro Dual-Luciferase Reporter Assay (Promega) was used to verify that the region 1 kb upstream of exon -2, which contains the presumed P2, can function as a promoter. The constructs with the sequence of P1 promoter exhibited the highest activity of luciferase (17, 82 ± 1,1 relative luciferase units) in HEP-G2 cells, while the P2 construct reached 3, 01 ± 0, 43 relative luciferase units. Four constructs carrying serial deletions of P2-containing construct pGL4 -353/-1509 were created to delineate the P2 promoter. The region from -1311 bp to -1509 bp (counted from the first ATG) likely contains a negative regulator of transcription activity.

Transcription initiation sites in the P2 were identified by 5'RACE. Three major initiation sites (328, 361 and 394 bp upstream of the first ATG) were identified. Although faint RT-PCR products, which may represent promoter upstream transcripts (PROMPTs), were amplified up to position -481 bp, quantitative RT-PCR using TaqMan probes (Applied Biosystems) confirmed that the -328, -361 and -394 positions are the main transcription initiation sites.

The expression of P2-originating transcripts measured by quantitative RT-PCR in twenty different tissues revealed similar levels relative to two known housekeeping genes, as well as to transcripts from P1. The P2 contains an unmethylated CpG island, multiple Sp-1 consensus binding sites and unlike P1 does not contain a TATA-box and CAAT boxes. Together with its expression pattern these features underscore the housekeeping character of the P2 promoter of GBA gene.

Supported by GAUK 121407, VZ MŠM ČR 0021620806

EFFICIENT CNS, PNS AND VICERAL GENE DELIVERY FOLLOWING FETAL AND NEONATAL INTRAVENOUS ADMINISTRATION OF AAV9 – AN APPROACH FOR STUDYING TYOE11 NEURONOPHATIC GAUCHER DISEASE

Ahad A. Rahim¹, Citra Mattar² Andrew MS. Wong³, Klemens Hoeffler³, Suzanne MK. Buckley⁴, Jonathan D. Cooper³, Jerry Chan² and Simon N. Waddington¹

¹*Institute for Women's Health, University College London, London, UK*

²*National University of Singapore, Singapore.*

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Introduction

The ability to efficiently deliver genes to the fetal brain represents a powerful research tool. A number of neonatal lethal neurodegenerative diseases present irreversible brain pathology during gestation. Therefore, neonatal intervention may be too late. Delivery of a therapeutic gene during gestation could provide answers to fundamental questions *e.g.* Does early intervention increase life expectancy compared to neonatal intervention? What percentage of cells needs to be transduced to have an effect? Which neural cells should we be targeting?

Aim

Recently, AAV9 was shown to cross the blood-brain-barrier following intravenous neonatal administration. We wanted to demonstrate this in fetuses and neonates in mice and non-human primates as a means of studying neonatal lethal diseases such as Type II Gaucher disease.

Results

In utero intravenous injection of single-stranded (ss) and self-complimentary (sc) AAV9 expressing GFP into mice (embryonic day 16) resulted in efficient global transduction of neurons in the CNS. Furthermore, there was a stark contrast in cell type transduction when compared to neonatal administration, confirmed by scanning confocal microscopy. The efficiency varied depending upon ss or sc configuration of the vector. Examination of injected mice by fluorescent microscopy, immunohistology and GFP ELISA revealed extensive and efficient transduction in the visceral organs in addition to muscle, bone, eye and skin. Gene expression was also seen in the peripheral nervous system. Similarly, extensive transduction was also observed following intravenous administration of AAV9 to the fetal macaque.

Discussion

The combination of CNS and visceral organ transduction is well suited to the study of specific lysosomal storage diseases such as Type II Gaucher disease where both CNS and visceral pathology require targeting and a suitable mouse model is available.

INFLUENCE OF c.(-203)A>G VARIANT IN THE GLUCOCEREBROSIDASE GENE IN TYPE 1 GAUCHER DISEASE PHENOTYPE

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The main cause of the Gaucher disease (GD) is due to mutations of the glucocerebrosidase (*GBA*) gene. However, wide phenotypic variability has been observed among patients carrying the same genotype. Therefore several factors may influence in phenotype, including polymorphic variants. We previously reported the presence of c.(-203)A>G (g.1256A>G) variant in exon 1 of *GBA* gene in Spanish GD patients. The aims of this study is to characterize of the function of this variant, and identify the frequency in GD patients vs controls, as well as to know the influence in GD phenotype Using restriction isotyping, electrophoretic mobility shift assay (EMSA), cell culture, transfections and luciferase assays, we conducted a systematic study in order to study this variant. We identified the variant in six of 300 normal control alleles and four of 412 alleles in GD patients, without statistically-significant differences in both groups. In the GD patients, the G-allele was always found in association with another mutation in the same allele. In the carriers of double mutant alleles the severity of disease was higher than non carriers of this polymorphism. EMSA assays showed a 35 % reduction in promoter activity containing the G-allele when was transfected into HepG2 cells. In conclusion, the c.(-203)A>G variant seems to be a functional polymorphism resulting in a decrease in the activity of *GBA* promoter. The change would be strong enough *per se* to elicit a GD phenotype. However, when this decrease is combined with an already defective *GBA* protein it might produce even lower levels of enzyme activity. This would be consistent with the more severe phenotype present in GD patients carrying the G allele.

DIFFUSION TENSOR IMAGING. STUDY OF BRAIN WHITE MATER IN PAEDIATRIC GAUCHER TYPE I AND TYPE III

Davies, E.H.¹, Seunarine, K.K.¹, Clark, C.A.¹, Vellodi, A².

¹*Institute of Child Health, University College, London.*

²*Great Ormond Street Hospital for Children NHS Trust, London*

Introduction

Markers of neurological dysfunction in Neuronopathic Gaucher Disease (NGD) are lacking. Diffusion tensor imaging (DTI) is a technique which allows us to study the microstructure of white matter brain.

Investigation of brain structure may offer better understanding of the pathophysiology and also generate a potential marker to monitor the effects of new emerging therapies for NGD.

Aim

The aim was to investigate the value of diffusion tensor imaging (DTI) to visualise and quantify white matter integrity in children with NGD.

Methods

DTI was performed on 4 NGD (1 boy, 3 girls, 12.2 years (± 3.5)) and 3 Type I patients (all boys, 9.5 years (± 3.3)). Fractional anisotropy (FA), mean diffusivity (MD), axial (λ_{axial}) and radial (λ_{radial}) diffusivity maps were calculated. Tract-Based Spatial Statistics (TBSS) were used to perform a voxel-wise statistical analysis of the main white matter structures.

Two separate control groups were selected to be age and sex matched for both the sub-cohorts as age and sex are known to be factors in the developing brain; Type III control group; 12.2 years (± 2.2) Type I control group; 9.7 years (± 2.7).

Data were acquired on a 1.5T Siemens Avanto scanner. 20 diffusion-weighted images were acquired at $b=1000$ s mm² along with 3 images at $b=0$ for normalisation. Images were processed using FSL to correct for eddy-currents, brain extraction and DTI reconstruction.

Results

The TBSS findings suggest the presence of microstructural white matter changes in NGD patients ($t > 3$, $p < 0.05$, uncorrected) primarily in the medial cerebellar peduncles with some differences in the right superior cerebellar peduncle compared to an age-sex matched control group with a decrease in FA and an increase in λ_{radial} and MD. There was also an increase in axial diffusivity; however the effect was much smaller than with other indices. In contrast, Type I patients compared to controls showed no significant difference in the cerebellar peduncles in the TBSS results ($p > 0.05$ uncorrected).

Discussion

This study provides new insight into NGD brain pathophysiology, and suggests that DTI may be an attractive surrogate marker, worthy of further exploration.

OSTEONECROSIS OF HEAD OF FEMUR IN GAUCHER DISEASE. A DESCRIPTIVE STUDY OF HISTOLOGICAL CHANGES

Ehud Lebel¹, Konstantin Reinus², Deborah Elstein³, Ari Zimran³, Gail Amir²

¹*Departments of Orthopedic Surgery and*

²*Pathology² and the*

³*Gaucher Clinic Shaare Zedek Medical Center, Jerusalem Israel*

Background

Osteonecrosis of the head of femur in patients with Gaucher disease is the most common and important problem necessitating orthopedic intervention. Osteonecrosis is commonly seen in the femur, tibia, humerus and tarsal/carpal bones. Damage to articular cartilage will inevitably lead to hip joint degeneration and a need for an arthroplasty in symptomatic patients. The nature of femoral head damage in Gaucher disease is not fully understood, and in general, there is very limited understanding of the pathology of bone necrosis in Gaucher disease.

Aim

This observational study describes histological findings in femoral heads of patients undergoing hip replacement.

Patients and methods

Our Gaucher clinic is arguably the largest center dedicated to clinical research and treatment of Gaucher disease. For the purpose of the current study we revised all histological material from femoral heads retrieved during surgical procedures at our institution (core decompression or hip arthroplasty). Background data was collected regarding severity of Gaucher disease, genotype, splenic status, duration of enzyme replacement treatment, and co-morbidities (skeletal and visceral).

Results

We re-evaluated 19 femoral head specimens of 15 patients with Gaucher disease. Signs of trabecular bone necrosis were seen in all specimens. Surprisingly, moderate-to-severe infiltration with Gaucher cells was seen in all specimens irrespective to the cumulative dose-years of enzyme replacement treatment. Regeneration of bone was seen in some cases, supporting the hypothesis that Gaucher bone is capable of regeneration even in the face of systemic disease and on top of severe marrow infiltration with Gaucher cells. Typical changes of osteo-arthritis were seen in some cases but were absent in others.

Conclusions

To our knowledge this is the first detailed histological study of osteonecrosis in Gaucher disease from both untreated and enzyme treated patients. This description highlights the following issues: that infiltration of Gaucher cells is prevalent in the femoral head despite long-term enzyme replacement therapy and that osteonecrosis is patchy and thus will not always lead to cartilage collapse. Osteonecrosis in Gaucher disease may be followed by re-growth of bone trabeculae.

HELPING TO OPTIMISE CARE IN GAUCHER DISEASE: A NOVEL ASSESSMENT AND MONITORING TOOL FOR THERAPEUTIC GOALS

Gregory Pastores on behalf of the Therapeutic Goals Taskforce group

Introduction

Clinical heterogeneity and variable disease progression are challenges to patient care in Gaucher disease (GD). Therapeutic goals provide a standardised approach to assessing and monitoring clinical outcomes.¹ A benchmark analysis evaluating 195 type 1 GD patients receiving enzyme replacement therapy (ERT) revealed that over half of patients had not reached all their therapeutic goals at four years.² There is also a large degree of variability in the awareness of therapeutic goals, with a rather limited use of the goals in the clinical setting.

Aim

This unmet medical need offers an opportunity to raise patient care standards through strategies advocating the use of therapeutic goals in day-to-day practice. The Therapeutic Goals Taskforce, a group of experts in GD in cooperation with the European Gaucher Alliance, aims to develop a solution; a novel, user-friendly, graphic point of care assessment and monitoring tool – the Therapeutic Goals MAP (Monitoring, Action and Progress).

Methods

The MAP, which will be available either as a hard copy or electronic version, is designed to be used in adults or children with type 1 GD and is adaptable for splenectomised patients. It unites clinical outcomes (in relation to disease severity) relevant to therapeutic goals in a visual, user-friendly format. Key patient information is collected at baseline. By plotting patient data against therapeutic goal domains at each patient visit over time, physicians can monitor attainment and maintenance of patient outcomes to make therapeutic decisions.

Results

The implementation of the MAP agreed by the Taskforce involves specific centres across Europe auditing the tool for ease of application in the clinical setting. The presentation will illustrate how the tool works using data from patient case studies (e.g. Figure 1).

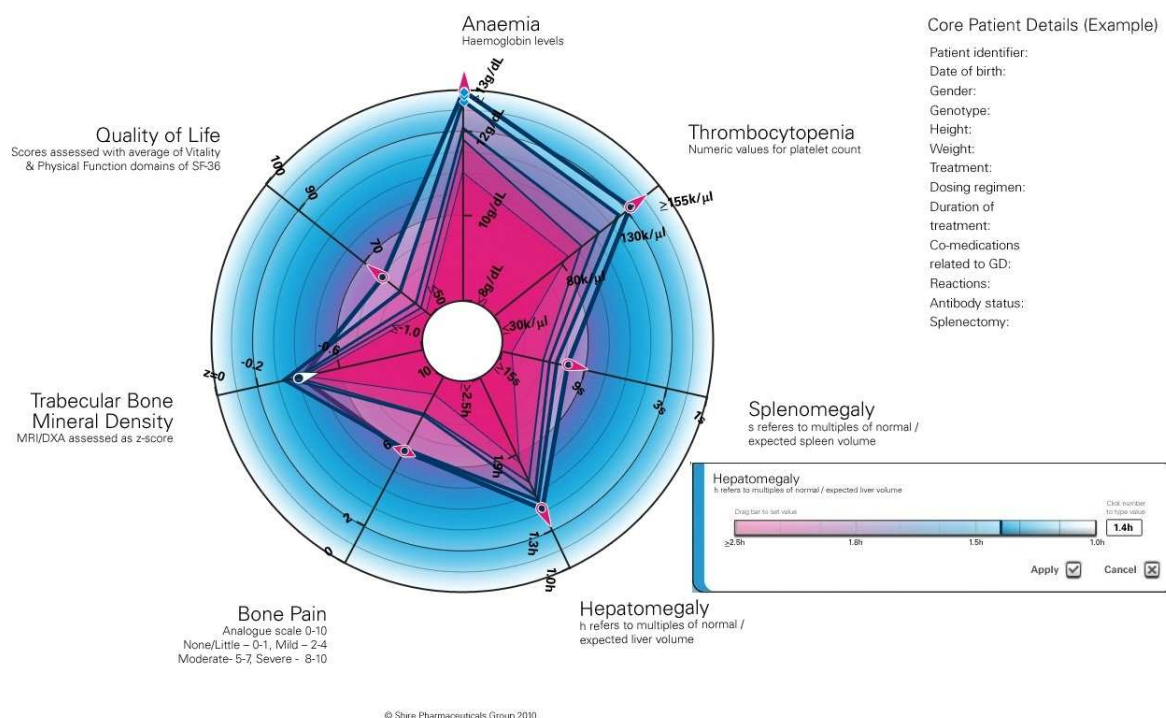
Discussion

The MAP represents a novel tool for monitoring therapeutic goals in clinical practice. It provides a standardised method for healthcare professionals, patients and families, to identify opportunities for improving care, creating a partnership in care. The MAP may also provide a standardised approach for benchmarking analysis and comparison of patient outcomes with different GD therapies.

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Therapeutic Goals 'MAP' tool for an adult patient (theoretical data)



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LONGTERM OUTCOME QITH ERT 26 GERMAN PEDIATRIC GAUCHER PATIENTS

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Introduction

11 years ago at the EWGGD meeting in Lemnos we reported about our treatment protocol “high-dose initial ERT followed by individual adapted maintenance dose” in 12 children with type 1 Gaucher disease. This report points out the follow up of the initial cohort and additional 14 children treated with the same treatment protocol.

Study design

A retrospective, one-centre cohort-study was performed. All patients were treated with the Mainz treatment protocol for children with symptomatic GD. ERT with imiglucerase/Alglucerase was timely initiated after diagnosis with a dose of 50-60 U/kg biweekly. When therapeutic goals would be achieved the absolute dose would not be changed even the child was gaining weight. As recently as chitotriosidase activity was 50% higher than the low mark, 400 U (one vial) more were given. Outcome measures include the therapeutic goals described by Pastores et al. 2004, last maintenance dose, the onset of major disease complication, the occurrence of normal bone marrow fat conversion during adolescence and chitotriosidase activity at the times of 2 years (t2), 5 years (t5), 10 years (t10) and 15 years (t15) on ERT.

Results

Twenty-six patients with non-neuronopathic GD in current age of 8-26 were included in this analysis. Age at initiation of ERT ranged between 1 year and 14 years. The average of follow-up was 9,8 years +/- 4,9 years. Therapeutic goals were achieved in all patients in one year and were still sustained (t2-t15). Bone complications were not observed. The average dose decreased from 56,6 +/- 7 U/kg/bw (t0) to 45,8 +/- 11 U/kg/bw (t2) to 36,8 +/- 13,6 U/kg/bw (t5) 32,2 +/- 10,8 U/kg/bw (t10) and 33,5 +/- 8,4 U/kg/bw (t15). Chitotriosidase activity dropped down from 10308 +/- 5813 nmol/ml/h (t0) to 482 +/- 407 nmol/ml/h (t2) 506 +/- 594 nmol/ml/h (t5), 524 +/- 620 nmol/ml/h (t10) and 805 +/- 850 nmol/ml/h (t15). T2- and T1-weighted MRI of the lumbar spine and/or the hips showed normal bone marrow fat conversions in the age of 14 to 22 years. Nobody developed inhomogenous bone marrow infiltration, which was called b-pattern from Poll and colleagues 2010.

Discussion

With the Mainz treatment protocol GD type 1 children with potentially progressive disease major disease complications were prevented. The maintenance dosages in this cohort are significantly lower than in German adult cohorts. We speculate that higher dosages in the initiation phase of ERT may allow lower dosages in the long term.

BONE MINERAL DENSITY RESPONSES TO ENZYME REPLACEMENT THERAPY IN PEDIATRIC PATIENTS WITH GAUCHER DISEASE

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Introduction

Skeletal involvement in Gaucher type I (GD1) disease is probably the most disabling aspect in adult patients and shows a slow response to enzyme replacement therapy (ERT). For paediatric patients, in which the disease tends to progress more rapidly than in adults, ERT may prevent future bone complications and may permit the achievement of a normal bone mineral density.

Methods

Eighteen paediatric GD1 patients (13 males, 5 females; mean age 10.4 years, range 3-16 years) were enrolled in the study. Patients received ERT for a period ranging from 4 to 16 years at an mean dosage of 32.3 U/kg/b.w. (range: 20 -60 U/kg). Lumbar (L1-L4) bone mineral density (BMD) was measured using a dual energy X ray absorptiometry, DEXA (Hologic QDR 1000 scanner then by Hologic Delphi QDR 4500); Z-score was analyzed in all the subjects.

Results

Seven patients (38.8%) showed a Z-score below the normal (≤ -2.0 s.d.) at baseline. A significant increase of Z-score value ($p=0.003$) was observed in all patients after 2 years of ERT. A normalization of the parameter was observed in 5 patients in course of therapy. When the modification of Z-score was analyzed in 6 patients who achieved the age of *peak bone mass* (25 years), it was observed the normalization of the value in 4 of them, while in the more severe ones (history of splenectomy and avascular necrosis) it remained pathologic: ≤ -2.5 s.d.

Discussion

The results show a significant BMD response to ERT after the first 2 years treatment. The achievement of an optimal bone mass during paediatric age, represents a clear vantage for adult life, reducing the risk bone complications in GD1 patients. Splenectomy and severe bone involvement in paediatric age seem to represent unfavourable prognostic factors for ERT response.

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SIGNIFICANT AND CONTINUOUS IMPROVEMENT IN BONE MINERAL DENSITY AMONG TYPE I GAUCHER DISEASE PATIENTS TREATED WITH VELAGLUCERASE ALFA: 69-MONTH EXPERIENCE, INCLUDING DOSE REDUCTION

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Introduction

Type 1 Gaucher disease (GD1) is associated with significant bone pathology. Velaglucerase alfa (Vα) was approved by the FDA for long-term enzyme replacement therapy in pediatric and adult patients with GD1. Zimran et al reported the safety and clinical activity from the Vα phase I/II studies (TKT025 & 025EXT) for treatment-naïve GD1 adults.

Aim

To evaluate long-term bone mineral density (BMD) changes in the intent-to-treat (ITT) population in TKT025EXT.

Methods:

TKT025 enrollment, skeletal surveys, and dual energy X-ray absorptiometry (DXA) established baseline (BL) skeletal pathology. Z-scores of the lumbar spine (LS) and femoral neck (FN) were analyzed over time. Clinical bone status at BL and 69 months was characterized (T-score: ≤ -2.5 =osteoporosis; > -2.5 , < -1 =osteopenia; ≥ -1 =normal [WHO criteria]).

Results

10 patients enrolled in TKT025EXT (4 men; 6 women; median age 35 yr (18–62)). At BL, all patients had GD1-related bone pathology; DXA Z-scores were (median [range]): LS -1.8 [-2.9 to -0.4], FN -1.5 [-2.9 to -0.2]; clinical status of LS and FN respectively were: 1 (10%) and 4 (40%) patients had osteoporosis, 8 (80%) and 5 (50%) had osteopenia, and 1 (10%) and 1 (10%) were in the normal range. Through 69 months, the average Vα dose was 40 U/kg; 4 of 10 patients were also treated with bisphosphonates. By 69 months, 2 LS and 1 FN osteopenic patients normalized and 1 FN osteoporotic patient became osteopenic; status change was only seen in the 6 Vα-only patients. BMD for the ITT population improved significantly by Months 24 (LS: 0.39; 95%CI 0.06, 0.72) and 33 (FN: 0.39; 95%CI 0.16, 0.62). In the linear mixed model, Z-scores were significantly lower than the reference population (LS y-intercept = -1.56 ; $P < 0.0001$) and FN (y-intercept = -1.42 ; $P = 0.001$). Both improved significantly over time (LS slope = $+0.13$ /year, $P = 0.002$; FN slope = $+0.08$ /year, $P = 0.001$). Among Vα-only patients, the Z-score LS y-intercept was -1.29 ($P = 0.005$), FN y-intercept was -1.24 ($P = 0.022$), and significant improvement was seen over time [LS slope = $+0.16$ /year ($P = 0.028$), FN slope $+0.10$ /year ($P = 0.006$)] Discussion: Vα was associated with clinically meaningful and statistically significant LS and FN BMD improvements as early as Month 24 (LS) and 36 (FN), despite dose reduction (60 to 30 U/kg during Year 2 of therapy) and significant BL skeletal pathology. The continuous improvement was not dependent upon continuous high-dose therapy, but was also seen with lower doses of Vα.

Vα is approved in the US; it is an investigational product in Europe.

EXPERIENCE IN AUSTRALIA & ISRAEL WITH IMIGLUCERASE SHORTAGE BY 'DRUG HOLIDAYS' OF ≤ 6 MONTH IN STABLE PATIENTS WITH TYPE I GAUCHER DISEASE

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Background

Because of an unanticipated shortage in global supplies of imiglucerase for Gaucher disease, two groups (in Australia and Israel) assessed the disease-specific parameters in cohorts that were unavoidably withdrawn from enzyme replacement therapy (ERT). At both sites, certain patients such as neuronopathic patients, children, and newly enrolled patients were not selected for ERT withdrawal. In Australia, ERT is administered and funded nationally (LSDP) and they sought advice from the Gaucher Disease Advisory Committee (GDAC) which monitors patients' progress, eligibility, and dosage regarding the outcome of off-therapy in Gaucher disease. In Israel, the largest Gaucher Clinic (Shaare Zedek, Jerusalem) unilaterally decided to monitor patients off-therapy ≤ 6 months before drug withdrawal and then re-tested these before/during reinstatement of (alternative) therapy.

Aim

In view of imiglucerase disruption to what is considered optimal dose therapy (in both sites, "low-dose"), with "drug holidays" for some patients, it was deemed important to monitor all off-therapy patients for any evidence of disease-specific deterioration.

Patients and methods

In Australia, 24/70 patients previously on ERT were put on a "drug holiday"; in Jerusalem 26/>200 patients previously on ERT were evaluated.

Results

In Australia, after 5 months no patient developed clinically detectable irreversible complication: 2/24 (8%) patients showed sufficient evidence of deterioration for ERT to be recommenced; 22/24 (92%) patients remained relatively stable despite deterioration in laboratory parameters. In Jerusalem, too, no irreversible complications were noted. Deterioration was seen in all four clinical parameters and in chitotriosidase activity in most patients; platelet counts were most sensitive to therapy withdrawal.

Conclusions

The overall impression of the results from both sources opportunistically on a "drug holiday" suggest it may be safe to temporarily withhold therapy, with associated

monitoring, in selected patients who have initially been “debulked” with ERT and show features of mild stable disease.

AZ receives consultancy fees from Shire HGT; receives consultancy fees and has options in Protalix Therapeutics and sits on its Scientific Advisory Board; and receives support from Genzyme Corporation for participation in the ICGG registry. GA has no conflicts of interest to report. DE receives consulting fees from Shire HGT.

SPANISCH EXPERIENCE WITH IMIGLUCERASE SHORTAGE

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In the last months an acute shortage of imiglucerase manufactured by the Genzyme Corporation (MA, USA) has occurred as a result of viral contamination firstly and other deficiencies in the production facility. In September 2009 a position statement based on the findings of the EWGGD and EGA, established a set of key recommendations about identification and monitoring of at-risk patients threatened. In Spain the follow-up of Gaucher disease (GD) patients and the strict complementation of rules of therapy have permitted to obtain a profile of the situation in a group of patients with restricted ERT. A total of 50 GD1 patients have been analyzed before and after 6 months of imiglucerase shortage. Have been excluded for analysis children in order to dose reduction has been minimal as well as patients who have switched to another ERT or miglustat therapy. Results: Gender: 25 males/25 females. Mean age of group: 45.3±15.3 (range:18-84) SSI at diagnosis(Dx): 8.7±3.8 (range:3-19) Chitotriosidase (CT) activity at Dx:13,383±12,783nM/mL.h; CCL18/PARC at Dx: 767±1,198 ng/mL. 20% of patients were splenectomized and 78% had bone disease at Dx. During shortage 23 patients (46%) discontinued therapy, in this period only one patient suffered a bone crisis and other anaemia as complications. Mean reduction of haemoglobin level: 2.7% (NS), platelet counts: 5.4% (NS). CT activity was increased 135% (p<0.03) and CCL18/PARC 8.2% (p<0.08). In 17 patients (34%) imiglucerase was reduced at 50%, in this period seven patients (41.0%) suffered a bone crisis and four patients (23.5%) required support therapy. Mean reduction of haemoglobin level 2.9% (NS), no changes in platelet counts. CT activity increase 48.2% (p<0.03) and not changes in CCL18/PARC concentration was observed. No significant changes in visceral size were observed. In addition, in 3 patients (6%) the reduction was 75% and 7 patients (14%) switched to another ERT (4 patients) or miglustat (3 patients). In conclusion, the shortage of imiglucerase in the last six months in this group of GD patients has produced a incidence in bone crisis of 20% and one case with anaemia and significant increase of CT activity and no significant changes in blood counts and CCL concentration, 14 % patients has required switch another therapy

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CLINICAL AND BIOCHEMICAL SURVEILLANCE PROGRAM IN A GROUP OF GD PATIENTS DURING SHORTAGE OF ERT DOSAGE.

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Introduction

Enzyme replacement therapy (ERT) with human recombinant beta-glucosidase has been available since 1996 without any dosage limitation until the first months of year 2009. In this period an acute productive crisis determined a reduction of enzyme availability for ERT. Consequently a piloted programme of dosage reduction has been prepared by manufacturing company in agreement with the clinician's community. The program has been set up to guarantee ERT maintenance for GD patients at risk to develop disease complications and who have not access to alternative treatments in Europe, the Middle-East and Eurasia areas. The major points of dose reducing guide-lines were:

- a) Maintenance of ERT access at regular dosage for patients in paediatric age;
- b) Reduction/suspension of ERT in adults, depending of their clinical conditions
- c) Guarantee of ERT access to GD symptomatic pregnant women.

Clinical consequences of ERT reduction/cessation have only been documented in small groups of patients: on the basis of current knowledge clinical deterioration may or may not occur when ERT is interrupted, but which patient will suffer a deterioration, if any, and to what extent, cannot be predicted.

Study aim

to detect clinical and biochemical deterioration in GD patients secondary to ERT shortage programme.

Methods

Thirty-four GD patients (32 GD1, 2 GD3, 16 female, 16 male; 31 adults, 3 children) were followed for 10 months after reduction (29 patients) or withdrawal (3 patients) of ERT. Biochemical parameters (Hb, leukocytes, platelets count, chitotriosidase) were collected every 3 months during follow-up. To register clinical modifications, patients were tested at least every four months with a quality-life questionnaire, mainly focused on exacerbation of bone symptoms.

Results and discussion

Statistical analysis of the data are in course and will be presented and discussed at the meeting.

IMIGLUCERASE SHORTAGE: THE FRENCH EXPERIENCE

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Introduction

The French Gaucher Disease Registry was implemented by the Referral Center of Lysosomal Diseases and the CETG with institutional support (INSERM, Institut National de Veille Sanitaire) and support from VML (Vaincre les Maladies Lysosomales), the patient association.

At the beginning of shortage, 530 Gaucher disease patients (pts) was registered, 230 pts received Imiglucerase (30 children and 10 type 3), 19 was treated with Miglustat. During the shortage a unique meeting was organized by the AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé) in March 2010. All partners were present: Referral Centers, CETG, VML, pharmaceutical firms (Genzyme, Actelion, Shire, Protalix/Pfizer).

Aim

The aim of the meeting was:

- To organize the best care and follow-up mainly for vulnerable pts
- To analyze the clinical and biological consequences in a sample of 20/115 untreated pts because of shortage.
- To make national recommendations and organize alternative treatments

Methods: Clinical and biological data of 20 pts followed and treated in the Referral was retrospectively analyzed (paired wilcoxon test).

Results

20 pts were analyzed: 9 female (45%), 19 type 1 and 1 type 3, median age of 39.9 years [28.2 – 82.6]. All type 1 patients stopped ERT between June and August 2010; only type 3 patient continued ERT with low doses (30 U/kg/15 days). ERT was prescribed between November 2009 and February 2010 in all pts. Median times of ERT interruption was 176 days [56 – 240 days]. One pt was better without ERT, 5 pts had no clinical symptoms, 9 had severe asthenia, 8 exacerbation of chronic bone pain, 4 abdominal pain, 2 hemorrhagic syndrome and 2 bone complications (one bone infarct and one pathological fracture).

Comparative analysis before and after shortage showed significant increase of median chitotriosidase (4900 nmol/mL/h [12 – 25750] vs 210 nmol/mL/h [30 – 1930], $p = 0.02$), decrease of median platelet count (130.000/mm³ [72.000 – 369.000] vs 156.000/mm³ [61.000 – 415.000], $p = 0,04$). Others biomarkers increase, but without significant results: median Angiotensin Converting Enzyme, 85 U/L [4 – 495] vs 46 U/L [13 – 105] $p = 0.2$; median Total Serum Ferritin, 225 ng/L [76 – 1250] vs 187 ng/L [39 – 858] $p = 0.07$; median Tartrate Resistant Acid Phosphatase level, 5 U/L [3 – 9] vs 3 U/L [1 – 6] $p = 0.06$.

Discussion

Imiglucerase shortage was responsible of clinical consequences. Biomarkers increase, but only significant for chitotriosidase.

UNITED KINGDOM EXPERIENCE OF 38 ADULT GAUCHER PATIENTS SWITCHED TO ENZYME REPLACEMENT THERAPY WITH VELAGLUCERASE AS A RESULT OF THE INTERNATIONAL IMIGLUCERASE SHORTAGE

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Introduction

The current approved treatments for Gaucher disease (GD) are enzyme replacement therapy (ERT) with imiglucersase[®] or oral substrate reduction therapy with miglustat[®]. ERT is indicated for long-term treatment in patients with a confirmed diagnosis of GD (b-glucocerebrosidase deficiency) and has been commercially available in Europe for over 15 years. An extended shortage of imiglucerase that began in June 2009 has necessitated that a large number of patients switch from imiglucerase to other therapeutic agents, including other replacement enzymes (velaglucerase and taliglucerase), which do not yet have EMEA marketing approval. No data are currently available regarding the clinical status of patients who have switched from commercial imiglucerase to other enzyme treatment in a clinical setting.

Aim

To report the approval process, baseline characteristics and preliminary follow-up for the administration of velaglucerase to 38 GD patients previously receiving other therapeutic agents at two national centers for GD in the UK.

Methods: Application for treatment of individuals with an unlicensed product was made to the MHRA on the basis of non-availability of the drug with marketing approval. Local approval to use velaglucerase was obtained from the drugs and therapeutics committees of the Royal Free and Addenbrooke's hospitals and patients gave informed consent to receive an unlicensed agent.

Results

38 patients with a confirmed diagnosis of GD switched from imiglucerase (35) or another investigational agent (3) to velaglucerase. 20 patients were splenectomised. A number of patients had received a reduction in their dose of imiglucerase prior to the switch. The median hemoglobin and platelet count prior to switch were 13.65 (range 10.9-16.2 g/dl) and 162 (range 34-441x10⁹/l) respectively. 20 patients have attended at least one follow up visit at median 127 days from switch (range 13-218 days) and have now recorded median hemoglobin and platelets of 14.15 (range 12.5-17.1 g/dl) and 150 (range 37-306x10⁹/l) respectively. All patients are currently receiving velaglucerase by home infusion therapy. There have been no significant adverse events.

Conclusion

We describe the combined experience of two UK centers in the switching of adult GD patients from imiglucerase to velaglucerase due to an international shortage of the licensed product. Use of velaglucerase in this context appears to be safe and effective and has enabled ongoing therapy of an increased number of GD patients and optimal use of existing supplies of imiglucerase.

GAUCHER DISEASE AND PARKINSONISM

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Introduction

Different lines of evidence demonstrate a relationship between mutations in glucocerebrosidase (*GBA*), the gene implicated in Gaucher disease, and the development of parkinsonism. Patients with Gaucher disease who are homozygous for mutations in *GBA* have a higher than expected propensity to develop Parkinson disease (PD). Furthermore, heterozygous family members of patients with Gaucher disease have an increased frequency of parkinsonism. Subsequently, screening cohorts of patients from centers around the world with parkinsonism for *GBA* mutations showed that subjects with PD as well as other Lewy body disorders had an increased number of carriers of *GBA* mutations as compared to age matched controls.

Aims

To elucidate the relationship between mutations in *GBA* and parkinsonism.

Methods

Complementary genetic, clinical pathology and cell biology approaches were

Results

A large multi-center collaborative study of cohorts with Parkinson disease explored. showed that the odds ratio for carrying a *GBA* mutation across different ethnicities is over 5.¹ Clinical evaluations of *GBA* mutation carriers with parkinsonism demonstrated a gamut of associated phenotypes ranging from classic L-dopa responsive PD to other forms of Lewy bodies disorders.² Neuropathology demonstrated both cortical and brain-stem Lewy bodies that stained positive for both alpha-synuclein and glucocerebrosidase. Biochemical analyses demonstrate oligomeric forms of alpha-synuclein in brain samples from subjects with parkinsonism and *GBA* mutations.

Conclusion

While the basis for this association has yet to be elucidated, evidence continues support the role of *GBA* as a Parkinson risk factor across different centers, synucleinopathies and ethnicities. Further studies of the association between Gaucher disease and parkinsonism will stimulate new insights into the pathophysiology of the two disorders, and will prove crucial for both genetic counseling of patients and family members and the design of relevant therapeutic strategies for specific patients with parkinsonism.

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EARLY SIGNS OF PARKINSON'S DISEASE IN GAUCHER PATIENTS

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Introduction

Several lines of evidence suggest an association between Parkinson's disease (PD) and mutations in the gene encoding the lysosomal enzyme glucocerebrosidase and heterozygous mutations in the Gaucher-gene were described as the most important risk factor for the development of an idiopathic Parkinson's syndrome. Detailed information about early PD symptoms in Gaucher disease (GD) patients are lacking.

Aim

We aimed at examine the frequency of subclinical symptoms of PD in a cohort of patients with genetically proven GD.

Methods

A detailed medical history of GD and PD specific symptoms as well as a thorough neurological examination including semi-quantitative smell-testing and a PD-score (UPDRS-III) was performed. Patients further underwent a neuropsychological examination, a cerebral magnetic resonance imaging (MRI) and a transcranial sonography (TCS) of the brain parenchyma.

Results

3 of the 13 examined patients had known PD for several years. None of the others had neurological symptoms suspicious of Parkinsonism. MRI did not show specific pathology in the basal ganglia in all patients. Neuropsychological testing revealed abnormalities of divided attention in all patients and disturbances of figural short term memory in women. TCS showed hyperechogenicity of the Substantia nigra in 9 of 12 patients examined, including the patients with known PD.

Discussion

Although clinically completely inconspicuous the majority of examined GD patients revealed sonographic results, known to be found in 90% of PD patients early in the course of the disease. These findings do not unequivocally prove PD in those patients, since hyperechogenicity of substantia nigra is also found in 10% of the normal population. GD patients should be monitored regularly by an experienced neurologist for PD symptoms to initiate symptomatic treatment as early as possible. Long-term follow-up studies are warranted to find out whether GD patients with substantia nigra hyperechogenicity are at a higher risk of developing PD compared to those without this abnormality.

FOUR FOLLOW UP OF TYPE III GAUCHER PATIENTS USING A MODIFIED SEVERITY SCORING TOOL

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Introduction

In 2007 The European Task Force for Neuronopathic Gaucher Disease (NGD) published a review of 55 cases of type III Gaucher disease. The only longitudinal clinical data available was Full Scale Intelligence Quotient (FSIQ). However FSIQ may not represent complete neurological status. The Severity Scoring Tool (SST) was developed in response to an unfulfilled need to reliably monitor the neurological disease of NGD and has subsequently been modified (mSST) to capture the severity of the 12 neurological domains.

Aim

Conduct sequential assessment of NGD patients using the mSST.

Methods

NGD patients in 3 countries (Poland, Germany, UK) were assessed sequentially, by the same assessors in each individual country. Enzyme replacement therapy (ERT) dose, Chito and FSIQ was also captured.

Results

39 patients were assessed. Time between assessments was 3.9 years (± 0.55). Mean age at follow up was 18.5 years. 69.2% were L444P homozygote.

ERT dose was reduced from 86 to 57IU/kg/2weeks lower ($p 0.002^*$) reflecting revised guidelines and current worldwide shortage of Cerezyme. Despite the reduced dose mean Chito was 1297nmol/per ml lower (not quite reaching significance $p 0.068$); possibly indicating that time on ERT rather than dose of ERT impacts levels.

The mean follow up mSST score was 7.1 (± 6.1) however mean mSST score differed across genotypes; 2.8 (± 0.8) in D409H and L444P; 6.1 (± 4.5) in L444P/ L444P and 13.5 (± 10.35) for the 'other genotypes'. Between groups ANOVA was significant ($p 0.019$).

The mean change in mSST score in the whole cohort was 1.7 (± 3.91) (highly significant $p 0.010$) but again mean change in mSST scores varied across genotypes; 1.1 (± 2.3) for the L444P homozygote group, 3.1 (± 8.2) for those with one L444P allele and 6.1 (± 6.2) for the 'other genotypes' cohort. There was no change in the D409H and L444P cohort.

Discussion

The mSST is useful to capture cross-sectional disease severity and to demonstrate disease progression over time. Results indicate that L444P homozygote and combined L444P and D409H alleles have a milder phenotype and progress less over time.

TYPE I GAUCHER DISEASE PATIENTS EXHIBIT COGNITIVE FUNCTION DEFICITS: RESULTS OF A TWO-YEAR PROSPECTIVE OBSERVATIONAL STUDY

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Background

The absence of neurological symptoms and signs is traditionally considered mandatory for a diagnosis of type 1 Gaucher disease (GD1), but in recent years many reports have emerged on central and peripheral neurological manifestations in GD1 patients. Despite the increasing number of reports, it has been unclear so far whether cognitive impairment is part of the disease. A multicentre, prospective study was undertaken to assess baseline neuropsychological status of GD1 patients, and to estimate progression of deficits.

Methods

Cognitive function was assessed in a large cohort of GD1 patients with the use of the Cognitive Drug Research (CDR) System, a validated computerized cognitive test system. Tests were done at baseline and every 6 months thereafter during a follow-up period of two years. Patients' data were Z-transformed and mean \pm SD GD1 population Z-scores were calculated by age-group.

Results

103 patients were enrolled of whom 102 attended at least one follow-up visit (median (range) age, 41.5 (18-75) years; disease duration 15 (0-56) years; 52% female). They were impaired relative to age-matched subjects on the composite scores Power of Attention (Z-score (mean \pm SD) -1.12 ± 1.52) and Speed of Memory (Z-score (mean \pm SD) -1.44 ± 1.49). No progressive decline in cognitive function was seen during the two-year follow-up period. Subgroup analyses revealed that age correlated with the composite scores Variability of Attention ($r_p = -0.359$, $p < 0.001$) and Quality of Working Memory ($r_p = -0.262$, $p < 0.01$). Correcting for age and the presence (or not) of polyneuropathy, severely affected patients (Zimran severity score ≥ 15 , $n = 13$) scored more poorly compared to mildly affected patients (SSI ≤ 5 , $n = 25$) on the composite measure Power of Attention (-2.15 versus -0.4 , difference -1.75 , 95% confidence intervals -0.24 & -3.25 , $p < 0.025$).

Conclusions

GD1 patients exhibit marked deficits to Power of Attention and Speed of Memory, reflecting an impaired ability to focus attention and process information, together with a slowing in the speed of retrieval of items from memory. Older age and more severe disease are associated with more severe deficits. We propose that these deficits are due to Gaucher disease itself.

NEURONOPATHIC GAUCHER DISEASE: Follow Up and Longterm Outcome in 30 German Patients

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Introduction

We studied genotype-phenotype correlation, disease progression, as well as morbidity and mortality of neuronopathic Gaucher disease (GD) by evaluating 30 patients diagnosed by us in the past 20 years.

Study design

A retrospective, one-centre cohort-study was performed. Our own medical records and those of co-operating hospitals were analyzed regarding manifestation, onset of neurological signs and symptoms, disease complications and mortality. All exons of the GBA gene were sequenced in 27 patients.

Results

Thirty patients (12 females/18 males) with neuronopathic GD were included in this analysis. Age at diagnosis ranged between 4 days and 32 years. Collodian baby manifestation was found in 2 newborns, died at the age of 8 and 32 days. Classical type II and III GD was diagnosed in 7 and 18 patients, respectively. The 3 remaining patients could not be clearly classified and were described as having an intermediate phenotype. All type II patients died within the first year of life. Remarkably, GD type II patients with progressive myoclonic epilepsy (PME) died considerably earlier (3-7 months) than those with pronounced bulbar signs but without PME (9–12 months). In patients with an intermediate phenotype, fatal bulbar complications and PME were observed at the age of 2-5 years. Of the 18 type III GD patients, 15 were still alive. Only a few of them showed signs and symptoms of mild neurological deterioration apart from supranuclear gaze palsy. Patients homozygous for the L483P (old terminology L444P) mutation had predominately visceral complications with marginal to moderate cognitive decline. Patients with L483P/D449H (L444P/D409H) had less severe visceral and neurological disease progression than those homozygous for L483P (L444P) or compound heterozygous for L483P (L444P) and another mutation but D449H (D409H). Patients from this latter group presented with a phenotype at the severe end of the type III spectrum with more rapid neurological deterioration and/or PME. Of the 8 patients with PME, 6 were compound heterozygote for the G241R (G202R) mutation.

Discussion

The Mainz cohort reflects the broad phenotypic spectrum of neuronopathic GD ranging from collodian babies to type III GD with predominant visceral disease. Overall mortality and morbidity due to visceral and neurological complications were substantial. In contrast to non-neuronopathic form, phenotype-genotype correlation was found in neuronopathic GD. PME predicted a poor outcome and was often associated with the G241R (G202R) mutation.

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PARKIN MEDIATED UBIQUITINATION AND DEGRADATION OF MUTANT GLUCOCEREBROSIDASE VARIANTS – A POSSIBLE LINK BETWEEN GAUCHER DISEASE AND PARKINSON DISEASE

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Mutations in the glucocerebrosidase-encoding gene lead to accumulation of glucosylceramides, 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.

We tested the possibility that the association between Gaucher disease and Parkinson disease reflects the fact that parkin is an E3 ligase of mutant glucocerebrosidase variants. Our results showed that the several mutant glucocerebrosidase variants, carrying the N370S, the L444P and the D409H mutations, associated with parkin. Normal parkin, but not its inactive mutants, affected the stability of the mutant glucocerebrosidase variants but not of the normal form. Parkin was involved in K48 mediated polyubiquitination of glucocerebrosidase mutants, indicating its function as an E3 ligase of mutant glucocerebrosidase variants. In transfected COS cells, parkin and mutant glucocerebrosidase colocalized in perinuclear-aggresome-like structures. Since mutant glucocerebrosidase variants present variable degrees of ER retention, we wondered whether the chemical chaperone Ambroxol can remove them from the ER, thus overcoming the need to ubiquitinate them for further elimination in the proteasome. Treatment of different Gaucher skin fibroblasts with Ambroxol led to an increase in the amount of mutant glucocerebrosidase variants that was removed from the ER. These results imply that Ambroxol may be considered as a treatment to avoid development of Parkinson disease in Gaucher disease patients or carriers.

THE LINK BETWEEN IMMUNITY AND GAUCHER DISEASE

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Introduction

Lipid engorged macrophages, the hallmark for Gaucher disease (GD), are not metabolically inert, but strongly express markers of activation via the alternative pathway, such as CCL18. Similarly, chitotriosidase, a chitinase secreted from alternatively activated macrophages is increased in plasma of GD patients. While these markers respond to therapy, they are not organ specific. Macrophages participate in innate immune response, along with others, including natural killer (NK) cells. In addition, they display plasticity, and change in response to environmental cues. In GD, substrate accumulation is suggested to be a major inducer of macrophages. The exact mechanisms of macrophage activation, however, are not known in GD. Although several lines of evidence, including inflammatory response, poor wound healing and insulin resistance, as observed in some patients with GD, may indicate that there are multiple pathways involved.

Aim

We hypothesized that in GD the mechanisms of macrophage activation is not limited to the alternative pathway. We explored the role of different elements of innate immunity including NK cell function, that controls macrophage function, and assessed response to therapy.

Methods

NK cell numbers and activity were evaluated in peripheral mononuclear cells (PBMC). NK cells are a subset of cytotoxic lymphocytes inducing cell death in tumor cells or virally-infected cells, and are characterized by CD16 and CD56 expression. In this assay the human erthroleukemia cell line, K562, was loaded with radioactive chromium and incubated with various ratios of PMBCs. Lysis of the K562 targets by NK cells was measured by the amount of chromium released into the supernatant. NK, NK T and CD8+ T cell numbers were determined by flow cytometry. Chitotriosidase levels were followed as a marker of alternative activation.

Results

The number of circulating NK cells was normal, but there was a significant decrease in target killing *in vitro*. Despite normalization of chitotriosidase with therapy, inflammatory markers in peripheral blood remained elevated.

Discussion

Macrophage activation pathways are heterogeneous in GD. NK cell dysfunction contributes to secondary macrophage activation, which may alter the response to therapy in some GD patients. Therapeutic interventions targeting macrophages may open new avenues for controlling the inflammatory component, which may have a role in skeletal, pulmonary and neurological complications of GD.

REDUCING GLYCOSPHINGOLIPIDS RESTORES INSULIN SENSITIVITY IN OBESSE MICE

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Introduction

Pharmacological lowering of (glyco)sphingolipids has been demonstrated to be a novel approach to improve glycemic control. We have recently shown that lowering of glycosphingolipids (GSL) including GM3 using the iminosugar AMP-DNM improved glucose homeostasis¹.

Aim

We wanted to address how this improved glucose homeostasis was regulated by focussing on GLS lowering effects in adipose tissue and liver.

Methods

Leptin-deficient obese (LepOb) mice were fed a commercial chow diet (AM-II) with or without 100 mg AMP-DNM/kg bodyweight per day for four weeks. Adipose tissue was analysed at the level of gene expression and by immunohistochemistry studying adipogenesis and inflammation in detail. Liver was analysed at the level of gene expression and by oil red O staining, focussing on inflammation and lipogenic -and glucose production pathways.

Results

In adipose tissue, a critical mediator in obesity-induced insulin resistance, we demonstrated that AMP-DNM restored insulin signalling, improved adipogenesis (characterized by increased expression of peroxisome proliferator-activated receptor (PPAR) α , insulin responsive glucose transporter (GLUT)-4 and adiponectin) and reduced pro-inflammatory adipose tissue macrophages (crown-like structures)².

In liver lowering of GSL reduced hepatic steatosis in obese mice. First, it was observed that insulin signalling was restored in liver. Second, liver weight -and fat content were reduced. Third, hepatic lipogenic -and glucose production pathways were inhibited by AMP-DNM. Last, inflammation was reduced by AMP-DNM³.

Discussion

Pharmacological lowering of glycosphingolipids by inhibition of glucosylceramide biosynthesis not only improves adipocyte function and inflammation in adipose tissue, but also reduces hepatic steatosis in obese animals.

1. Aerts et al. 2007. *Diabetes*. 56:1341-1349.
2. Van Eijk et al. 2009. *PLoS ONE* 4:e4723
3. Bijl et al. 2009. *Hepatology* 50:1431-1341

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ROLE OF GBA2 IN MORBUS GAUCHER

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Background

GBA2, synonym for bile acid beta-glucosidase, is a highly conserved, membrane bound enzyme of the endoplasmic reticulum. GBA2-deficient mice accumulate glucosylceramide in different tissues (Yildiz et al. JCI 2006).

The most common inherited defect in glycosphingolipid breakdown is M. Gaucher, arising from mutation in the gene encoding the lysosomal acid- β -glucosidase (GBA1). GBA1 and GBA2 are both glycolipid hydrolases and catalyse the same reaction: The hydrolysis of glucosylceramide to glucose and ceramide. Despite this common function they do not share any sequence identity and expressed in different sub cellular compartments.

Patients with Gaucher disease show no clear correlation between phenotype and genotype, the striking biochemical similarities between GBA1 and GBA2 might indicate an important role of GBA2 in the pathogenesis of Gaucher disease.

Methods

Therefore we investigated in different primary fibroblasts from M. Gaucher patients with known different *GBA1* mutations the enzyme activity, mRNA expression of *GBA2* and *GBA1*. Furthermore we designed a gene assay for several *GBA2* SNPs and analysed 350 DNA samples from M. Gaucher type I and 60 DNA samples type II/III.

Results

Fibroblasts from some severe affected Gaucher patients, show beside the decreased *GBA1* mRNA level also a decreased *GBA2* mRNA level, and no *GBA2* enzyme activity could be detected compared to the control fibroblasts. Furthermore westernblot analysis from these samples showed a significantly decreased *GBA2* protein level. On the contrary, fibroblasts with L444/L444 or NS370S/NS370S mutation with an existing *GBA2* enzyme activity show no difference in *GBA2* protein level compared to control human fibroblasts. However the gene assay comparison between controls and M. Gaucher type I and type II/III does not show any significant association.

Conclusions

We conclude that *GBA2* gene does not have a modifying effect for M. Gaucher type I and type II/III. But it remains unclear why some severe affected M. Gaucher patients show a significant decreased *GBA2* protein and *GBA2* mRNA level which needs to be further investigated.

GBA1 DEFICIENT MICE RECAPITULATES GAUCHER'S DISEASE DISPLAYING SYSTEM-WIDE CELLULAR AND MOLECULAR DYSREGULATION BEYOND THE MACROPHAGE: EVIDENCE FOR OSTEOBLASTIC BONE FORMATION DEFECT UNDERLYING OSTEOPENIA

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In Gaucher's disease (GD), GBA1 gene mutations result in the deficiency of glucocerebrosidase and the accumulation glucocerebroside, in the lysosomes of macrophages. This metabolic defect leads to a complex phenotype involving visceral organs, bone marrow and the skeleton. The prevailing macrophage-centric view, however, does not explain emerging aspects of the disease, such as cholesterol gallstones, malignancies, autoimmune diathesis, and osteoporosis, all of which appear to be resistant to macrophage-directed enzyme replacement therapy.

To understand the pathophysiology of the multi-system involvement in GD, we conditionally deleted the GBA1 gene in the hematopoietic and mesenchymal cell lineages using an Mx1 promoter.

We fully recapitulated human GD. GD1 mice exhibited a striking, up to ~60- and ~30-fold accumulation of glucocerebroside (GL1) in the liver (mean: ~15-fold) and the spleen (mean: ~10-fold), respectively. Concomitantly glucosylsphingosine (LysoGL1), was elevated: compared to GL1, there was a dramatic and earlier temporal elevation of LysoGL1 up to 166-fold (mean: ~30-fold) and 60-fold (mean: ~23-fold) in liver and spleen, respectively. Expression microarray analysis revealed that hepatomegaly (with associated liver enzyme elevation and low HDL cholesterol) and splenomegaly was associated with 5 to 50-fold elevation in the expression of 296 and 95 genes respectively, representing immune response, signaling, apoptosis, cell cycle and lipid metabolism pathways. Serum concentrations of numerous cytokines were strikingly elevated: IL-1 β , IL-1 α , IL-6, MIP 1 α , MCP-1, and TNF- α were all consistent with the increased macrophage population. In addition there was significant elevation of serum concentrations of IL-2, IL10, GM-CSF, IFN- γ , IL-3, IL-9, MIP 1 β and IL-13, presumed to be derived from specific T-cell subsets likely involved in GD1. Indeed flow cytometry of the thymus, spleen and lymph nodes was notable for a striking impairment of thymic T cell maturation, in addition to the expected increase of macrophage populations.

Unexpectedly, we found severe osteoporosis in our GD mice arose from a defect in osteoblastic bone formation that was due to inhibition of protein kinase C (PKC) that was mediated by the accumulation of LysoGL1 (but not of GL1). Micro-CT revealed significant decrements in trabecular volume, with evidence of trabecular thinning and loss, accompanied, on calcein labeling, with reduced mineral apposition rates in GBA1 mice compared with control littermates. This was associated with reduced osteoblastoid colony forming units, CFU-ob, in bone marrow stromal cell cultures, consistent with an autonomous defect in osteoblast maturation. Contrary to the prevailing view, osteoclastic

resorption *in vivo* and TRAP-positive osteoclast formation *ex vivo* remained unaffected in GBA1 deficiency.

Together our study provides a clear demonstration for the involvement of cell lineages other than macrophages and LysoGL1-mediated inhibition of PKC in the pathophysiology of GD. Important therapeutic implications may eventually follow from these studies.

IMIGLUCERASE BIOSIMILAR DEVELOPMENT FOR GAUCHER DISEASE

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Introduction

ISU302 has been developed as biosimilar therapeutics to Cerezyme® (imiglucerase). Imiglucerase has provided greater life-long benefits to Gaucher patients and their families, albeit big burden of treatment costs. The healthcare system as well as patients tremendously needs an alternative with much reasonable cost. ISU Abxis' expertise in cell-line and production process development has succeeded in securing a biocomparable molecule to imiglucerase. In the aspects of the structural, physiochemical and biological characterization, ISU302 have shown excellent similarity to Cerezyme. ISU302 has also demonstrated the efficacy through *in vitro* studies and, in toxicity studies, has well tolerated in a similar mode to imiglucerase. Based on the analytical and preclinical studies, ISU302 met the similar quality and efficacy with Cerezyme®.

Aim

Biosimilar development with higher productivity and equivalency with Cerezyme®.

Methods

The stable CHO cell-line producing imiglucerease (ISU302) was prepared and established to cell banking system for further production. The drug substance was produced through the perfusion culture mode in using disposable bioreactors and its carbohydrate structure was engineered to mannose exposed forms during purification steps. The drug substance was characterized in the aspects of structural, physicochemical and biological comparability with Cerezyme®. The toxicity and efficacy performances of in-vitro test and animal test were conducted

Results & discussion

The productivity of ISU302 was achieved higher than those of the original drug, which permits a reasonably economic production cost. Furthermore, the analytical and preclinical results of drug substance demonstrate its therapeutic, quality and safety equivalent with the original product in the aspect of comparability. Therefore, ISU302 would be provided to the patients and physicians with an alternative treatment option with cheaper price than the original product. Currently, the phase I and III clinical trial has been initiated in Korea and multi-countries. This study will evaluate that the comparable safety and efficacy of ISU302 to Cerezyme®

NOVEL ENZYME REPLACEMENT THERAPY FOR GAUCHER DISEASE: III Pivotal Clinical Trial with Plant Cell Expressed Recombinant Glucocerebrosidase (prGCD) - taliglucerase alfa

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Background

Taliglucerase alfa is a carrot-cell-expressed recombinant human β -glucocerebrosidase for treating Gaucher disease produced by Protalix Biotherapeutics. A Phase III double-blind, randomized, parallel dose groups (60units/kg /infusion and 30units/kg /infusion) clinical trial was completed.

Methods

A 9-month, 20-infusion clinical trial (NCT number: 00376168) was based on inclusion/exclusion criteria in treatment-naïve adult symptomatic patients. Safety endpoints were drug-related adverse events and antibody formation. Primary efficacy endpoint was reduction in spleen volume (as measured by MRI). Secondary endpoints were: change from baseline of: hemoglobin levels, liver volume, and platelet count. Exploratory parameters included biomarkers and QCSI imaging.,

Results

Patients from 11 centers worldwide were enrolled and were equally randomized to each dose group. No serious adverse events were reported; drug-related adverse events were mild/moderate and transient. Two patients (6%) developed IgG, none of each having neutralizing activity, two other patients developed hypersensitivity reactions. There was a statistically significant difference from baseline for the primary efficacy measures, i.e splenic reduction ($p < 0.0001$) in both treatment groups. The primary endpoint was achieved already after six months of treatment in both treatment groups. Statistically significant improvements compared with baselines were observed in the secondary endpoints, including increase in hemoglobin level, decrease in liver size and increase in

platelet count at the 60 U/kg dose. Statistically significant improvements compared with baselines were observed in hemoglobin level and liver size and significant nominal elevation in platelet count in the lower dose of 30 U/kg.

Conclusions

Taliglucerase alfa 30 and 60 units/kg administered by intravenous infusion for 9 months in 31 patients with moderate to severe Gaucher disease demonstrated to be safe and efficacious in a clinically relevant and statistically robust manner. Additional clinical trials including an extension study, switch over study from imiglucerase to taliglucerase, early access program and pediatric study are currently on-going

ENZYME REPLACEMENT THERAPY WITH VELAGLUCERASE ALFA SIGNIFICANTLY IMPROVES KEY CLINICAL PARAMETERS IN TYPE 1 GAUCHER DISEASE: POSITIVE RESULTS FROM A RANDOMIZED, DOUBLE-BLIND, GLOBAL, PHASE III STUDY

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Introduction

Type 1 Gaucher disease, resulting from deficiency of glucocerebrosidase, leads to anemia, thrombocytopenia, hepatomegaly, splenomegaly and skeletal abnormalities. The current gold standard treatment is enzyme replacement therapy (ERT) using recombinant glucocerebrosidase manufactured in Chinese hamster ovary cells. Velaglycerase alfa is a new ERT being investigated for use in type 1 Gaucher disease. Unlike recombinant enzymes, it is produced by gene activation in a human cell line.

Aims

To evaluate the efficacy and safety of velaglycerase alfa in type 1 Gaucher disease.

Methods

Twenty-five treatment-naïve, anemic, type 1 Gaucher disease patients (age range 4–62 years) were randomized to intravenous velaglycerase alfa 60 U/kg (n=12) or 45 U/kg (n=13) every other week for 12 months.

Results

At 12 months, mean hemoglobin concentration increased in both groups (60 U/kg: 23.3% increase, $+2.4 \pm 0.3$ g/dL, $P < 0.0001$; 45 U/kg: 23.8% increase, $+2.4 \pm 0.4$ g/dL, $P = 0.0001$), as did mean platelet count (60 U/kg: 66% increase, $+51 \pm 12 \times 10^9/L$, $P = 0.0016$; 45 U/kg: 66% increase, $+41 \pm 14 \times 10^9/L$; $P = 0.0111$). Mean spleen volume decreased in both groups (60 U/kg: 50% decrease, $-1.9 \pm 0.5\%$ body weight, $P = 0.0032$, from 14.0 multiples of normal [MN] at baseline to 5.8 MN; 45 U/kg: 40% decrease, $-1.9 \pm 0.6\%$ body weight, $P = 0.0085$; from 14.5 to 9.5 MN) as did liver volume (60 U/kg: 17% decrease, $-0.8 \pm 0.3\%$ body weight, $P = 0.0282$, from 1.5 to 1.2 MN; 45 U/kg: 6% decrease, $-0.3 \pm 0.3\%$ body weight, $P = 0.3149$, from 1.4 to 1.2 MN). Velaglycerase alfa was generally well tolerated with no drug-related serious AEs, and no patient withdrew due to an AE. The most common AEs were headache, nasopharyngitis, injury, arthralgia, cough, and pyrexia. A single patient developed antibodies.

Conclusions

In this global, multicenter study, velaglycerase alfa 60 U/kg and 45 U/kg was generally well tolerated and effective as a first-line treatment for adults and children with type 1 Gaucher disease. All clinical parameters measured demonstrated clinically meaningful improvements after 12 months, with a greater response seen with velaglycerase alfa 60 U/kg.

Velaglycerase alfa is approved in the U.S. and is investigational in the EU.

WHOLE BODY MRI IN TYPE I GAUCHER PATIENTS: PRELIMINARY RESULTS OF BONE INVOLVEMENT

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Introduction

Skeletal disease effects major morbidity in the majority of type I Gaucher patients. [1]. In previous studies (2,3) the assessment of skeletal disease was performed predominantly by MRI either in the lower extremities and/or in the lumbar spine using different scoring systems. New MR scanners have been established in clinical routine meanwhile that allow whole body investigations in one hour.

Aim

To evaluate the bone involvement of the entire skeletal system in type I Gaucher patients using whole body MRI and compare different scoring systems of bone involvement in the same patient.

Methods

Whole body MRI was performed in 41 adult type I Gaucher patients on a 1.5T superconducting magnet (Avanto, Siemens) using dedicated whole body coils with total imaging matrix (TIM) technology. In 2 patients follow-up was performed. A standard protocol without contrast media was performed in all patients using coronal T1-, T2-weighted (thighs) and STIR-sequences of the whole body, sagittal T1-, T2- (lumbar spine) and STIR-sequences of the entire axial skeleton as well as axial T2-weighted sequences of the chest and the entire abdomen. Bone marrow involvement was analyzed by Düsseldorf-Gaucher-Score (DGS), bone marrow burden score (BMB), vertebra-disc-ratio (VDR) and the morphological pattern of involvement determined by homogeneous type A or non-homogeneous Typ B.

Results

Whole body MRI was well tolerated in all patients. In patients with severe bone involvement (type B, high DGS and BMB) humeral bone involvement with avascular necrosis was detected more frequently than in patients with lower DGS and BMB and type A morphology. The morphological pattern of bone involvement was comparable in cervical, thoracic and lumbar vertebral bodies.

There was a correlation between type B, high DGS and BMB. In one female patient an incidental renal cell carcinoma was detected. In another patient partial regression of a hepatic gaucheroma was documented under enzyme replacement therapy.

Discussion

Severe bone involvement in the lumbar spine and the lower extremities seem to be associated with a severe humeral involvement and bone complications. Whole body MRI is a promising and feasible technique to assess the entire skeletal system and should be implemented in routine clinical diagnostic algorithms in type I Gaucher patients.

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ANTIGENIC DIFFERENCES IN PATIENTS WITH TYPE I GAICHER DISEASE RECEIVING VELAGLUCERASE ALFA OR IMIGLUCERASE ENZYME REPLACEMENTS THERAPY IN CONTROLLED CLINICAL TRIALS

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Introduction

In type 1 Gaucher disease, ERT with human acid beta-glucosidase can reverse most disease symptoms. However, development of antibodies to therapeutic proteins may impact patient safety, efficacy and drug pharmacokinetics.

Aim

To compare antibody responses in patients receiving velaglucerase alfa or imiglucerase.

Methods

Patients participating in the velaglucerase alfa Phase III studies (TKT032, TKT034 and HGTGCB-039) were monitored for development of anti-drug antibodies (ADA). Of 99 patients treated, 82 received velaglucerase alfa and 17 received imiglucerase. Calibrated, sequential, direct bridging electrochemiluminescence (ECL) methods were developed and validated for velaglucerase alfa and imiglucerase. Samples positive in the bridging assay were confirmed and isotyped using quantitative radioimmunoprecipitation (RIP) assays (for IgG ADA) or indirect binding ECL immunoassays (for IgE, IgA and IgM). Isotype-specific goat-human hybrid controls were developed for the latter. All ADA-positive samples were tested for neutralizing antibodies (NAb) using methods based on in vitro inhibition of enzyme activity. All patient specimens were tested for ADA in parallel in a masked fashion.

Results

ADA-positive cut-points for both therapeutics were established as 5 and 4 ng/mL in the ECL bridge and RIP assays, respectively. In study TKT032, 25 naïve patients received velaglucerase alfa for 12 months; only 1 patient developed IgG ADA and NAb in response to velaglucerase alfa. In study TKT034, 40 patients previously treated with imiglucerase, received velaglucerase alfa for 12 months. No patient became anti-velaglucerase ADA-positive, despite the 3 patients having had pre-existing anti-imiglucerase ADA at baseline. In study HGT-GCB-039, 17 naïve patients were treated with velaglucerase alfa and 17 naïve patients with imiglucerase for 9 months, with identical doses. No velaglucerase alfa-treated patient developed any ADA, 4 imiglucerase-treated patients developed IgG ADA (of whom 1 developed NAb) in response to imiglucerase.

Discussion

Highly sensitive and equivalent methods were developed, standardized and validated to directly compare patient antibody response to velaglucerase alfa and imiglucerase. Seroconversion was seen in 1% of patients treated with velaglucerase alfa and in 23% of patients treated with imiglucerase, suggesting significant antigenic differences between velaglucerase alfa and imiglucerase.

Velaglucerase alfa is approved in the U.S.

SAFETY AND EFFICACY OF VELAGLUCERASE ALFA IN GAUCHER DISEASE TYPE I PATIENTS PREVIOUSLY TREATED WITH IMIGLUCERASE

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Aims

To examine the safety and efficacy of velaglucerase alfa every other week in patients with type 1 Gaucher disease previously receiving imiglucerase.

Methods

This global open-label, 12-month study enrolled 41 patients ≥ 2 years of age to receive velaglucerase alfa at a dose equal to their prior imiglucerase dose, with infusions administered over 1 hour every other week.

Results

Forty patients received study drug (18 male, 22 female; 4 previously splenectomized; age range, 9–71 years, 25% <18 years). Median prior imiglucerase use was 67 months (range 22–192 months). Velaglucerase alfa doses were: 15–22.5U/kg (n=14), 22.5–37.5U/kg (n=12), 37.5–52.5U/kg (n=7), and >52.5U/kg (n=7). Velaglucerase alfa was generally well tolerated with most AEs of mild or moderate severity. Overall, 11 of 40 patients (28%) experienced an AE considered possibly or probably related to study drug; the majority of these events were considered infusion related. No patient experienced a life-threatening AE. One severe adverse event was considered probably related to treatment: a patient had a severe hypersensitivity reaction during the first infusion, and chose to discontinue the study. This patient tested negative for all 4 isotypes (IgE, IgM, IgG, IgA), including neutralizing antibodies, both at the time of the infusion and 2 weeks later. Throughout the study, no patients developed IgG antibodies to velaglucerase alfa. Clinical parameters were sustained at therapeutic levels through 1 year (table).

	n	Baseline median	Mean change or % change from baseline to month 12	90% CI	Clinically significant cutoffs
Hemoglobin concentration (g/dL)	40	10.8	-0.1	-0.3, 0.1	-1, 1
Platelet count (x10⁹/L)	40	162	7.0%	0.5%, 13.5%	-20%, 20%
Normalized liver volume (% of body weight)	40	1.9	0.0%	-2.6%, 2.6%	-15%, 15%
Normalized spleen volume (% of body weight)	36	0.5	-5.6%	-10.8%, -0.4%	-15%, 15%

Conclusions

Adult and pediatric patients with Gaucher disease, previously treated with imiglucerase for ≥30 months, were successfully transitioned to velaglucerase alfa, with stability in clinical disease measures over 12 months.

velaglucerase is approved in the U.S. and is investigational in the EU.

A NOVEL, ULTRA-SENSITIVE TECHNIQUE TO VISUALIZE ACTIVE GLUCOCEREBROSIDASE

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Gaucher disease is the most prevalent lysosomal storage disorder, in which glucosylceramide accumulates due to deficiency of the lysosomal glucosylceramidase (GBA1). Carriership for Gaucher disease has recently been identified as a major risk in Parkinsonism. Presently no method exists to visualize active GBA1 molecules *in situ*. We report the design, synthesis and application of fluorescent, activity-based probes, allowing highly specific labelling of enzymatically active GBA1 molecules *in vitro*, *in situ* in cultured cells and mice *in vivo*. As starting point we used a known irreversible GBA1 inhibitor, cyclophellitol, which mimics glucosylceramide. GBA1 activity was abolished by binding covalently to glutamic acid residue E340 in active GBA1, with cyclophellitol being one-hundred-fold more potent than the commonly used GBA1 inhibitor conduritol β-epoxide. Via a spacer, hydrophobic BODIPY-moieties were attached to cyclophellitol, creating two avid probes for labelling of GBA1 molecules (named Inhibody Green and Inhibody Red). We demonstrate that the inhibition and labelling with these probes is another hundred-fold more potent when compared to cyclophellitol and truly activity-based. Detection of *in vitro* labelled recombinant GBA1 on slab gels following electrophoresis is in the low attomole range for both probes. We present evidence by FACS analysis, fluorescence (time-lapse) microscopy and pulse-chase experiments of highly efficient labelling of GBA molecules in intact cells as well as in tissues of living mice. In addition, the use of the fluorescent probes to study inhibitors and tentative chaperones in living cells is illustrated. In conclusion, we have currently developed two highly potent activitybased fluorescent probes for labelling of GBA1 molecules in mammalian cells and tissues, which allow for versatile research.

ELIGLUSTAT TARTRATE, AN INVESTIGATIONAL ORAL COMPOUND FOR GAUCHER DISEASE TYPE 1 (GD1): PHASE 2 RESULTS AFTER 2 YEARS

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Introduction

Eliglustat tartrate (Genz-112638) is a novel, oral inhibitor of glucosylceramide synthase under clinical investigation for the treatment of GD1.

Aim

Report the efficacy and safety of eliglustat tartrate after 2 years of treatment.

Methods

This ongoing, open-label, uncontrolled, multicenter, Phase 2 clinical trial of eliglustat tartrate (50 or 100 mg bid depending on plasma level) enrolled 26 untreated adults with GD1. Efficacy results included changes from baseline in spleen and liver volumes, hemoglobin and platelet levels, bone mineral density (BMD), and other skeletal findings (reviewed centrally), and achievement of GD1 therapeutic goals for anemia, thrombocytopenia, and organomegaly.

Results

For the 20 patients who completed 2 years of treatment, mean hemoglobin level increased by 2.1 ± 1.5 g/dL and platelet count by $81.5 \pm 56.0\%$; mean spleen volume decreased by $52.4 \pm 10.7\%$ and liver volume by $23.9 \pm 12.8\%$. No bone crises or reductions in mobility were reported. Available femur MRI showed improved dark marrow signal in 8 patients and stability in 10 others. There were no new lytic lesions or bone infarcts. Existing lytic lesions remained stable; of 7 existing infarcts, 1 improved and 6 remained stable. Lumbar spine BMD improved continuously: DXA Z-scores improved by 0.060 ± 0.69 and DXA T-scores by 0.56 ± 0.78 . After 2 years of treatment, most patients had met published therapeutic goals for 2 years of treatment (Pastores et al. 2004); more patients met hemoglobin and spleen volume goals (95% and 100%, respectively) than liver volume or platelet count goals (65% for both). Eighteen of 20 patients (90%) met at least three of four therapeutic goals. Eliglustat tartrate was well tolerated. The most common adverse events (AEs) through 2 years were viral infections (6 patients), and urinary tract infections, increased blood pressure, and abdominal pain (3 patients each). Eight drug-related AEs, all mild, occurred in 6 patients.

Discussion

Eliglustat tartrate has shown promising efficacy and safety as a potential oral substrate-reduction therapy for GD1 with continued improvements in hematologic, visceral, and bone parameters after 2 years. Two controlled Phase 3 registration studies are underway in untreated patients (ENGAGE) and in patients switching from enzyme replacement

therapy (ENCORE). A third Phase 3 study will compare different dose frequencies of eliglustat (EDGE).

Pastores G et al. *Semin Hematol.* 2004;41(suppl 5):4–14.

SP²-IMINOSUGARS AS PHARMACOLOGICAL CHAPERONES FOR GAUCHER DISEASE: MUTATION PROFILING, CELLULAR UPTAKE AND INTRACELLULAR DISTRIBUTION STUDIES

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Introduction

Bicyclic sugar-shaped glycomimetics that incorporate a bridgehead nitrogen atom with substantial sp² character (sp²-iminosugars) behave as competitive inhibitors of glycosidases with tuneable selectivity patterns.¹ The rigid bicyclic framework imposes a restricted orientation of the hydrophobic substituent that is probably responsible for the unprecedented selectivity among human lysosomal enzymes,² making them very good candidates for chemical chaperone therapy.³

Aim

To evaluate the potential of sp²-iminosugars as chemical chaperones for different mutations of Gaucher disease (GD) in comparison with NN-DNJ and to investigate cell internalization and mutant protein rescuing mechanisms.

Methods

sp²-Iminosugars were synthesized from readily available sources. The chaperone activity was determined in Gaucher fibroblasts, including F213I/F213I, G202R/L444P, N188S/G193W, N370S/N370S, F213/L444P, L444P/RecNcil and L444P/L444P mutants. A fluorescent inhibitor was designed for cell internalization and distribution studies.

Results

Amphiphilic sp²-iminosugars exhibited much higher chaperone activities than NN-DNJ in F213I/F213I, G202R/L444P, N188S/G193W and F213/L444P mutants. Internalization occurred through passive diffusion and the chaperone colocalized with the mutant protein at the lysosomes.

Discussion

We have developed a general approach for the synthesis of bicyclic sp²-iminosugar very well-suited for molecular diversity-oriented strategies. The chaperone effects were generally stronger than those observed for NN-DNJ, suggesting that these compounds are promising candidates for clinical treatment of GD patients, especially for neuronopathic forms. This seems to be related to variations on binding affinity at neutral and acidic pH, a situation that emulates the environment at the ER and the lysosome, respectively. The sp²-iminosugars have been shown to be internalized through passive diffusion, rescue the mutant protein and further shed from fibroblasts into the surrounding

medium, an important characteristic of promising chaperones in view of clinical applications to avoid accumulation-derived toxicity.

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**THERAPEUTIC STRATEGIES FOR GAUCHER DISEASE:
DIFFERENT THERMOSTABILITY OF VELAGLUCERASE ALFA AND IMIGLUCERASE**

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Gaucher disease (GD) is a disorder of glycosphingolipid metabolism caused by deficiency of lysosomal acid β -glucosidase (GC) activity, due to conformationally or functionally defective variants, resulting in progressive deposition of glycosylceramide in macrophages. The aims of this work has been to compare imiglucerase (the enzyme currently used for replacement therapy) obtained from CHO cells vs velaglucerase alfa (a novel therapeutic enzyme form, recently approved by FDA) obtained from human cultured fibroblasts in terms of conformational stability and enzymatic activity. Both enzymes have been assayed at two pH values: neutral pH, reflecting the endoplasmic reticulum environment, and mildly acidic pH, reflecting the lysosomal environment by using Differential Scanning Calorimetry and enzyme activity assays with 4-methylumbelliferyl- β -D-glucopyranoside. Our results reveal that, although velaglucerase alfa and imiglucerase exhibit very similar activity profiles (the temperature for maximum enzyme activity around 46 and 43 °C, respectively). Both enzymes were more stable at lysosomal than at endoplasmic pH values: T_{ms} 54.5 °C and 50.5 °C for imiglucerase and T_{ms} 55.2 °C and 50.7 °C for velaglucerase alfa. So velaglucerase alfa shows higher *in vitro* thermal stability and is less prone to aggregation/precipitation.

In conclusion, imiglucerase undergoes aggregation around 50 °C, which does not occur to velaglucerase alfa. A considerable lower unfolding enthalpy for imiglucerase at pH 7 was observed, which could be related to the R495H substitution or its glycosylation pattern.

P02

PREIMPLANTATION GENERIC DIAGNOSIS (PGD) FOR A TREATABLE DISORDER: GAUCHER DISEASE TYPE I AS A MODEL

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Introduction

Preimplantation genetic diagnosis (PGD) is a technique that enables identification of unaffected embryos prior to in vitro fertilization (IVF) transfer in couples at risk for a Mendelian disorder. Most cases involve severe genetic diseases with neurological features and/or major malformations.

Aim

We present two couples in which PGD was performed for prevention of type 1 Gaucher disease, a non-neuronopathic, non-lethal disorder.

Material and Methods

We developed a multiplex fluorescent PCR protocol, simultaneously amplifying the familial mutations and eight closely spaced, highly polymorphic informative microsatellite markers surrounding the gene, to be used for PGD analysis.

Results

Couple #1 mother was homozygous for the N370S mutation and the father carried the 84GG mutation; their first daughter receives specific Gaucher therapy. One PGD cycle resulted in seven embryos of which four had the paternal wild type allele; two were transferred resulting in a healthy baby boy born at term. Couple #2, each a carrier (N370S and R359Q), whose first-born child had died (age 5 years) of Gaucher disease, underwent 8 PGD cycles. The last cycle resulted in a clinical pregnancy and is currently at 4 weeks.

Discussion

PGD is an effective and accurate method for preventing Gaucher disease type I in carrier couples. Since this disease is treatable, special ethical considerations and careful selection of couples should be performed.

GAUCHER DERIVED MONO-LAYERES PRIME PLASMA CELLS FOR SURVIVAL AGAINST CHEMO-THERAPEUTIC AGENTS

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Background

Gaucher disease (GD) is caused by a deficiency in lysosomal glucocerebrosidase resulting in accumulation of glucosyl ceramide in reticuloendothelial cells and dysfunction of the monocytic lineage. Clinical manifestations include bone marrow failure, organomegaly and skeletal pathology. GD patients have a higher incidence of multiple myeloma and benign gammopathy and anecdotal reports suggest GD patients with myeloma are more refractory to chemotherapy. Prior published work has established the supportive nature of macrophages/osteoclasts from non-Gaucher patients on co-cultured multiple myeloma cell lines in relation to proliferation, survival and drug resistance to chemo-therapeutic agents. However this has not been evaluated in GD patients.

Aims

To demonstrate a proliferative or survival advantage of plasma cells grown on adherent mono-layers generated from GD patients compared to controls.

Methods

Peripheral blood monocytes were grown in the presence of RANK-L and M-CSF for 14 days prior to the addition of either NCI-H929 or U266 plasma cells for a further 7 days. Harvested day 21 plasma cells were subjected to cell cycle analysis (BrdU/PI), viability testing (Trypan blue exclusion and AnnexinV/PI positivity) and MTT assay (melphalan IC₅₀ at 24 hours). Trans-well inserts were also used to contact deprive co-cultured NCI-H929 plasma cells. PARP cleavage was determined by western blot of lysates from harvested NCI-H929 plasma cells 24 hours post addition of 50µm Melphalan. Additionally western blotting was performed on lysates of harvested NCI-H929 plasma cells for pro- and anti-apoptotic proteins (BIM, PUMA, MCL-1, BCL-2, BCL-XL, STAT1, STAT5 and ERK).

Results

Based on the mean percentage of plasma cells in S-phase GD derived mono-layers did not convey a proliferative advantage for either the NCI-H929 (control 29.9±6.0%; Gaucher 32.43±2.2%) or U266 plasma cell line (control 27.93±6.0%; Gaucher 26.92±1.8%). There was no significant difference in viability as assessed by trypan blue exclusion or annexinV/PI assay of either plasma cell line harvested from control co-cultures compared to those grown on GD derived mono-layers. However the melphalan IC₅₀ was elevated in plasma cells harvested from GD patients compared to controls for both the NCI-H929 (Mean Control=41.58µM; Gaucher=54.64µM; p<0.01) and the U266 cell lines (Mean Control=78.21µM ; Gaucher=110.9µM; p<0.05). Increased apoptosis was confirmed by enhanced PARP cleavage from harvested NCI-H929 plasma cells derived from control mono-layers post-exposure to melphalan. Paired experiments utilising trans-well inserts demonstrated that the protective effect of GD derived mono-layers was contact dependent (Mean Contact IC₅₀=48.18µM; Transwell IC₅₀=34.97µM; p<0.01). Western blot analysis demonstrated an increase in BIM and BCL-XL in untreated NCI-H929 plasma cells harvested from control co-cultures but there was no difference in the levels of PUMA, MCL-1, BCL-2, STAT1, STAT5 or ERK.

Conclusion

(1) GD derived adherent mono-layers do not convey a proliferative or survival advantage over controls but do lead to an increase in melphalan IC₅₀ which is contact dependent

(2) GD derived monocytic cultures prime plasma cells differently compared to controls. Further experimental work is required to elucidate the mechanisms behind the observed increased in gammopathy in GD patients.

P04

ERAD OF THE L444P MUTANT GLUCOCEREBROSIDASE VARIANT

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Gaucher disease is an autosomal recessive lysosomal storage disorder caused by mutations in the gene encoding the lysosomal enzyme glucocerebrosidase. The most prevalent among type 1 patients and among Jews, which has been exclusively associated with this form of the disease, is the N370S missense mutation. The most prevalent mutation among non-Jewish patients, carried by 38% of these patients, is the L444P. It is a severe mutation that in homozygosity leads to neuropathic type 3 Gaucher disease. It was shown that mutant glucocerebrosidase variants present variable degrees of ER retention and undergo ER associated degradation (ERAD). However, ERAD of the L444P glucocerebrosidase variant has never been characterized. We tested the ERAD of this mutant. Our results indicate that the L444P mutant protein undergoes extensive ERAD. The amount of the L444P mutant protein was markedly decreased in comparison to normal glucocerebrosidase, and at least 50% of it was localized to the ER, as tested by endonuclease H sensitivity and confocal microscopy. We have also shown that the L444P glucocerebrosidase variant undergoes polyubiquitination and proteasome dependent degradation and therefore it is stabilized upon proteasome inhibition.

Recently ambroxol, a known expectorant, was identified as a putative pharmacological chaperone for mutant glucocerebrosidase. We tested the effect of ambroxol on L444P homozygous fibroblasts. Our results indicated that this chaperone increases the amount of lysosomal L444P mutant glucocerebrosidase with a concomitant increase in its enzymatic activity.

DENDRITIC CELL DYSFUNCTION IN PATIENTS WITH GAUCHER DISEASE

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Introduction

Recent studies suggest the existence of an increased incidence of autoimmune complications and hematological malignancies in patients with Gaucher disease (GD). The mechanism for these phenomena is unclear. The current study investigates immunological functions in GD patients, focusing on dendritic cells (DCs) function, depending on enzyme replacement therapy (ERT).

Patients and Methods

Fifteen GD patients; 8 naïve and 9 on ERT (Imiglucerase, Cerezyme®), and 6 healthy volunteers, were studied (IRB approval number - 2920). Monocytes and DCs, obtained from PB, were analyzed for quantitative and functional properties.

Results

Study results are summarized in Table 1. The number of monocytes and DC precursors (BDCA1, BDCA2) appeared to be decreased in naïve GD patients. However, in patients receiving ERT, these parameters were almost normal. BDCA1 cells, and monocyte-derived immature DCs (iDCs), cultured with an autologous serum, showed a significantly reduced uptake capacity, which tended to normalize when iDCs were cultured with an AB serum. Furthermore, antigen uptake in patients receiving ERT was also higher than that observed in naïve GD patients. Similarly, T cell stimulation capacity of patient's derived mature DCs, cultured in an autologous serum, was significantly impaired, reflected by a decreased interferon gamma (IFN- γ) secretion and a reduced T cell proliferation capacity. Interestingly, the IFN- γ secretion, but not the T cell proliferation capacity, was found to be normal in GD patients treated with ERT.

Table 1:

	Serum	Healthy	ERT	GD
Monocytes	•	$16 \cdot 10^6$	$8.5 \cdot 10^6$	$21 \cdot 10^6$
BDCA1	•	$2.3 \cdot 10^6$	$0.9 \cdot 10^6$	$2.4 \cdot 10^6$
BDCA2	•	$6 \cdot 10^6$	$2.8 \cdot 10^6$	$8.7 \cdot 10^6$
Uptake	A	%39	11%	%17
	A	%36	%25	%21
IFN- γ secretion **	A	%7	4%	10%
	A	%7	%7.6	10%
Tc proliferation **	A	x 8	X 4	X 2.9
	A	x 8	X 4.4	X 2.8

* Uptake by Monocyte-derived iDCs

** T cell response to stimulation with mature DCs compared with T cell alone

Conclusions

The current results suggest the existence of quantitative and functional impairment of DCs in GD patients, reflected by a decreased number of DCs precursors, a low uptake capacity of iDCs and a decreased T cell stimulatory capacity. DCs functional abnormalities are at least partly caused by serum-derived components, as demonstrated by the significant improvement in functional capacities when using AB rather than patient's serum. It is noteworthy that DCs impairments are at least partly corrected by ERT.

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PERIPHERAL NEUROPATHY IN ADULT TYPE 1 GAUCHER DISEASE: A TWO-YEAR PROSPECTIVE OBSERVATIONAL STUDY

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Background

Type 1 Gaucher disease is currently categorised as non-neuronopathic, although recent studies suggest peripheral neurological manifestations. We report prevalence and incidence data for peripheral neuropathy and associated conditions from a multinational, prospective, longitudinal, observational cohort study in patients with type 1 Gaucher disease, either untreated or receiving enzyme replacement therapy.

Methods

The primary outcome parameters were the prevalence and incidence of polyneuropathy, evaluated by standardised assessments of neurological symptoms and signs, and electrophysiological studies. All diagnoses of polyneuropathy were adjudicated centrally. Secondary outcome parameters included the prevalence and incidence of mononeuropathy, other neurological or electrophysiological abnormalities not fulfilling the criteria for a mono- or polyneuropathy, and general type 1 Gaucher disease symptoms. Furthermore, a literature search was performed to identify all studies reporting on prevalence and incidence of polyneuropathy in the general population.

Results

103 patients were enrolled (median (range) age, 42 (18-75) years; disease duration 15 (0-56) years; 52% female); 14 (13.6%) were untreated and 89 (86.4%) were on enzyme replacement therapy. At baseline, 11 patients (10.7%; 95%CI 5.9,18.3) were diagnosed with sensory motor axonal polyneuropathy. Two (1.9%; 95%CI 0.1,7.2) had a mononeuropathy of the ulnar nerve. The two-year follow-up period revealed another six cases of polyneuropathy (2.9 per 100 person-years; 95%CI 1.2,6.3). Patients with polyneuropathy were older than those without ($p < 0.001$). Conditions possibly associated with polyneuropathy were identified in four patients only, being monoclonal gammopathy, vitamin B1 or B12 deficiency, folic acid deficiency, non insulin-dependent diabetes mellitus, renal insufficiency and alcohol abuse. The eleven cases of polyneuropathy found at baseline were confirmed during follow-up. According to the literature, the prevalence of polyneuropathy in the general population was estimated between 0.09 and 1.3%, and the incidence was estimated between 0.0046 and 0.015 per 100 person-years.

Conclusions

The prevalence and incidence of polyneuropathy in patients with type 1 Gaucher disease is increased compared with the general population.

P07

IRON METABOLISM IN TREATMENT-NAÏVE GAUCHER PATIENTS IN RUSSIA

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Introduction

Gaucher disease is characterized by proliferation and dysfunction of tissue macrophages that play keystone role in different metabolic and immune processes including maintenance of iron homeostasis.

Aim

Characterize iron metabolism in treatment-naïve adult type 1 Gaucher patients.

Methods

The group included 71 treatment-naïve patients (18–75 yrs, mean age 34 yrs): 24 males and 47 females. Diagnosis was confirmed by enzyme assay. Normo- or hypochromic anemia was found in 35 (49%) patients. We investigated serum indices of iron metabolism: serum iron (SI), total iron binding capacity (TIBC), transferrin iron saturation (TIS), ferritin, transferrin. The control group consisted of 35 healthy volunteers.

Results

Level of SI was normal in 80% patients, decreased – in 14%, increased – in 6%. Mean level of SI was 17 $\mu\text{mol/L}$ (N 10-28). Ferritin was increased in 82% patients, normal – in 18% patients, mean level was 980 mkg/L (N 40-300). TIS was normal in 56% patients, decreased – in 31%, mean level was 26% (N 20-40). Transferrin was normal in absolute majority of patients, mean level 2,57. The complex review of blood count and serum indices of iron metabolism showed that absolute majority (82%) of patients with normal or decreased Hb had hyperferritinemia with normal or decreased SI and TIS. The signs of iron overload (elevated SI and TIS) were revealed in 4 (6%) patients, 2 of them had heterozygous β -thalassemia, 1 – heterozygous mutation (C282Y) of HFE gene (inherited hemochromatosis). All 4 patients with iron overload had chronic viral hepatitis C (HCV-RNA+) with low activity. Iron deficiency (decreased SI, serum ferritin and TIS) was found in 3 (4 %) patients with hypochromic anemia. According to clinical signs, posthemorrhagic iron-deficient anemia was diagnosed in all these patients.

Discussion

Iron metabolism in treatment-naive adult Gaucher patients is characterized by signs of inflammatory changes. In anemic patients these changes are called “anemia of chronic disorders”. True iron deficiency was very rare in our group of patients and was seen only in 3 patients with recurrent bleedings. Iron overload was associated with the presence of other molecular abnormalities (iron metabolism and erythropoiesis) and chronic HCV infection.

P08

MEASUREMENT OF SPLEEN AND LIVER VOLUMES USING MRI IN AN ENZYME-REPLACEMENT THERAPY MULTI-CENTER TRIAL ON NAIVE PATIENTS WITH GAUCHER DISEASE

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Gaucher Disease is characterized by a lack of beta-glucocerebrosidase activity causing the accumulation of glucocerebroside in key organs such as spleen, liver, lungs, bones and sometimes brain. Enzyme Replacement Therapy consists in providing an exogenous active enzyme to degrade the accumulated substrate in the macrophages and reduce the volume of the affected organs. Spleen and liver volume changes are common endpoints used for efficacy evaluation.

A Phase III randomized double-blind parallel-group dose-ranging clinical study involving 11 international sites recruited 31 naïve Gaucher patients to show the efficacy of a plant cell derived recombinant enzyme, taliglucerase alfa, in reducing spleen and liver volumes after 9 months of treatment. Accurately measuring organ volumes along the study was therefore paramount for detection of response to treatment.

Patients underwent abdominal MRI examinations at Screening, Months 6 and 9. Transverse T2, T1 and In/Out-of-phase, and coronal T1 sequences were performed, using standardized settings across sites. Spleen (resp.liver) volume was semi-automatically delineated on each T2 (resp. T1 transverse) slice using a 3D automatic unsupervised bayesian segmentation followed by manual correction by experienced technologists using advanced editing tools. The other sequences gave additional insight in patient's anatomy. All MRI volumetric analyses were made by independent and blinded imaging experts. Screening data were read by 1 reader for eligibility assessment. Data of all randomized patients were submitted to 2 readers for efficacy evaluation.

For each volume, the variability between readers was computed. Out of 178 spleen and liver volumes measured twice, the mean variability was 0.30% for spleen and 0.53% for liver. Adjudication due to high variability was not required.

This method was found to be precise in monitoring spleen and liver volume changes over time, with a much lower variability than traditional methods. The majority showed a variability of less than 1%, supporting the accuracy of the results. Given the observed minimal variability rates among multiple readers, a single read of each volume would be sufficient to detect response to treatment.

P09

A CYROMETRIC STUDY OF THE RED BLOOD CELLS IN GAUCHER DISEASE SUGGESTS THAT THEY MAY BE INVOLVED IN INCREASED ERYTHROPHAGOCYTOSIS

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Background

Glucosylceramidase-deficient macrophages transform into pathogenic Gaucher cells following the phagocytosis of red blood cells (RBCs) and subsequent accumulation of glucosylceramide (GlcCer). This phenomenon likely occurs in Gaucher disease (GD), as exemplified by the finding of intramedullary erythrophagocytosis further indicating abnormal RBC-macrophage interactions. We hypothesized that the erythrophagocytosis observed in GD may be at least partly due to abnormalities in the RBCs themselves.

Methods

To investigate this hypothesis, we used flow cytometry FSC/SCC to study RBCs sampled from seven patients with GD in term of their shape and expression of markers of senescence and phagocytosis. Cells from two of the seven patients were evaluated before and nine months after the start of enzyme-replacement therapy (ERT).

Results

Four untreated patients were found to have abnormal flow-cytometric profiles suggesting an alteration of Gaucher RBC morphology. Scanning electron microscopy confirmed this finding by revealing many abnormally shaped RBCs. While there was no evidence of desialylation of membrane glycoconjugates or phosphatidylserine exposure, RBCs viability (calcein-AM test) and CD47 expression were reduced. These anomalies found in RBCs sampled from two patients prior to ERT were no longer present after a nine-month long ERT.

Conclusions

We report on previously overlooked alteration of Gaucher RBCs that may facilitate erythrophagocytosis in untreated patients. These erythrocytic anomalies may take part in the abnormal crosstalk between RBCs and macrophages leading to the accumulation of pathogenic Gaucher cells in GD. Their potential role in the anemia, the excess of aggregation and rheological anomalies associated with GD must now be addressed.

DETERMINANTS OF PERSISTING THROMBOCYTOPENIA IN PATIENTS WITH TYPE I GAUCHER DISEASE TREATED WITH ALGLUCERASE/IMIGLUCERASE FOR 4-5 YEARS

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Introduction

Thrombocytopenia in type 1 Gaucher disease (GD) may result in surgical, obstetrical and spontaneous bleeding. Treatment with alglucerase/imiglucerase generally results in rapid improvement in platelet count but, in rare cases, the platelet response to therapy is slow or lacking.

Aim

To identify patient characteristics associated with (and potentially predictive of) persisting thrombocytopenia despite therapy by retrospective analysis of ICGG Gaucher Registry data.

Methods

1,016 type 1 GD patients with an intact spleen, date of diagnosis and therapy initiation, no treatment breaks 4-5 years after therapy initiation, and known platelet counts, were classified into four groups by last platelet count 4-5 years after therapy initiation: $\geq 120 \times 10^3/\mu\text{L}$ ($n = 772$); ≥ 100 to $<120 \times 10^3/\mu\text{L}$ ($n = 94$); ≥ 80 to $<100 \times 10^3/\mu\text{L}$ ($n = 80$); and $<80 \times 10^3/\mu\text{L}$ ($n = 70$, of which 20 had $<60 \times 10^3/\mu\text{L}$ platelets/ μL). Patients were characterized by demographics, BMI; platelet count; anemia; biomarkers; hepatomegaly; splenomegaly; and skeletal assessments at baseline (therapy initiation) and after 4-5 years of therapy. Initial enzyme dose and cumulative average dose were noted. Possible associations with persisting thrombocytopenia were tested using a multivariate analysis calculating odds ratios adjusted for age at diagnosis and therapy initiation, genotype, sex, year of diagnosis, and year of therapy initiation.

Results

Of the 1,016 patients in this study, 20 (2%) had platelet counts $<60 \times 10^3/\mu\text{L}$ after 4-5 years of therapy. These patients all had severe splenomegaly at baseline. Associations between persisting thrombocytopenia were found between baseline low platelet count ($<80 \times 10^3/\mu\text{L}$), splenomegaly and anemia ($p < 0.0001$). After 4-5 years, persisting thrombocytopenia was associated with anemia ($p < 0.0001$), reduced WBC ($p =$

0.049), splenomegaly ($p < 0.0001$), hepatomegaly ($p = 0.006$), bone pain ($p = 0.035$) and cumulative enzyme dose (dose range 18.6-55.5 U/kg/2weeks; doses $< 25^{\text{th}}$ percentile: $p = 0.043$). Too few data prevented reliable analysis of other bone parameters and biomarkers.

Discussion

Persisting thrombocytopenia after 4-5 years of therapy is rare. Strong associations between persisting thrombocytopenia and splenomegaly, low platelets and anemia at baseline suggest a relationship with advanced spleen involvement, possibly with fibrosis. Findings underline the importance of initiating treatment before irreversible complications have developed.

MUTATIONS FREQUENCIES IN GAUCHER'S DISEASE IN DIFFERENT POPULATIONS

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Introduction

Gaucher's disease is the most common lysosomal storage disorder. It is due to reduced or deficient lysosomal glucocerebrosidase (β -glucosidase) activity, which leads to storage of glucocerebroside within lysosomes. The clinical presentation is extremely heterogeneous reflecting the underlying mutations in the GBA gene.

Aim

This study has looked at frequencies of both common and rare mutations in different populations of patients with Gaucher's disease.

Methods

Genotypes have been assigned by DNA analysis and sequencing of the entire coding region of the glucocerebrosidase gene using an ABI3130 genetic analyser.

Results

106 British patients of non-Jewish origin have been analysed showing the following mutation frequencies: L444P = 27%, N370S = 25%, R463C = 9%, Recombinant alleles = 7.1 % while rare mutations account for 25%. Phenotype classification show 74 patients (70%) with Type I non-neuronopathic disease (adult onset n = 35 and childhood onset n = 39), where the most frequently seen alleles are N370S (35%), L444P (13.5%) and R463C (13%). Of 32 patients showing neuronopathic disease, Type II n = 22 show a high frequency of rare mutations or unknown alleles (52%) often with L444P (42%), while Type III patients n = 10 show a high frequency of L444P (75 %). In the Egyptian population studied n = 15, the most common mutation is L444P (63%), with 53% of these patients showing Type III disease. Similarly in the Middle Eastern population n = 24, L444P accounts for 65% of mutant alleles observed and 71% of patients presented with Type III disease. The Asian population n = 10 show 33 % L444P with 70% rare mutations.

Discussion

The most frequently seen allele in all populations studied is L444P. The UK population shows a high frequency of rare mutations contributing to a higher incidence of both Types I and II disease than observed in Egyptian and Middle Eastern populations studied. The UK has a significant population of Asian origin, however the number of Asian patients referred for genotyping is relatively few. Our centre has a many Asian patients with MPS I, MPS IV and Juvenile Sandhoffs, suggesting that Gaucher is not highly prevalent in the Asian gene pool. Novel mutations identified will be presented.

A MODIFIED SEVERITY SCORING TOOL FOR NEURONOPATHIC GAUCHER DISEASE

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Introduction

Clinical monitoring of the neurological manifestations of *Neuronopathic Gaucher Disease* (NGD) is difficult. A Severity Scoring Tool (SST) was developed in 2007 to meet this gap and is now part of the revised European recommendations on the management of NGD. However further development was needed, for clinical use and to be in line with E9 Statistical Principles for Clinical Trials guideline (International Conference on Harmonisation) should the tool be used in clinical trials.

Aim

Complete SST validation; through evaluating inter and intra rater agreement, 'weighting' of individual domains to reflect impact on disease severity and exploration of the mean important clinical difference.

Methods: Six NGD patients were assessed on the same day by 2 separate assessors and 9 NGD patients assessed sequentially by one assessor. Seven international experts collaborated in 2 separate web based nominal group technique to identify a 'weighting' for all domains and the change in score needed to be regarded as clinically meaningful.

Results

Inter rater agreement was good as assessors were in 100% agreement for four of the eleven domains and in agreement 83% of the times for another three. Poor agreement was noted in three domains. Intra rater agreement was evident, despite the variable clinical presentation of the patients. Sequential assessments of patients demonstrated the sensitivity of the SST to capture change in disease presentation. Experts ranked Epilepsy and Pyramidal signs as contributing the most severity in NGD. Cerebellar Ataxia, Horizontal Gaze Palsy and Ophthalmology were ranked as having the least impact in disease. Domain scores were 'weighted' to reflect this, and the SST modified accordingly. A group consensus was reached on the 'weighting' of all domains and an addition of a 12th domain included in response to identify meaningful clinical difference.

Discussion

While identifying the mean important clinical difference, experts noted that it was difficult to do this without accounting for the impact of age on disease. "Age" as a stand alone domain was considered as a twelfth domain but later modified to "Age at onset of seizures" (weighted according to age-brackets). The Severity Scoring Tool has now been modified (mSST) and offers a validated tool for the assessment of neurological manifestations in NGD.

QUANTIFYING ATAXIA IN NEURONOPATHIC GAUCHER DISEASE

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Introduction

Neuronopathic Gaucher Disease (NGD) is an inherited lysosomal disorder. Clinical features include horizontal gaze palsy, epilepsy and ataxia, however there is great heterogeneity. There are currently no validated surrogate markers of disease.

Aim

To evaluate the use of a modified Severity Scoring Tool (mSST) and GAITRite walkway.

Methods

Nine NGD patients were studied and 5 Type I Gaucher children served as a control group.

Results

The mean age for NGD cohort was 10.2 years (± 4.1) and 10.6 years (± 3.4) for the Type I cohort. Five of the NGD cohort was L444P/L444P. Mean mSST score for NGD cohort was 6.3 (± 5.3) but with a large spread, and 0.2 (± 0.4) for Type I cohort. Gait parameters explored were Velocity (cm/s), Cadence (Steps/minute), Step Time (cms), Step Time (seconds), Single and Double Support (% of gait cycle), Base of Support (cms). Direct group comparison identified a statistical significance in Velocity ($p0.005$), Step Time ($p0.044$), Single Support ($p0.044$) and Double Support ($p0.040$). This significance remains when Z scores generated from LMS centiles are utilised. LMS centiles summarise the changing distribution of a normal population by three curves representing the median (M), coefficient of variation (S) and skewness (L). Cadence Z score becomes statistically significant ($p0.046$). mSST correlates with Step Time, Single and Double Support $r0.690$; -0.562 ; 0.597 respectively. Pyramidal domain within mSST correlated highest with gait parameters.

Discussion

Deviations in the NGD gait profile suggest that the patients reduce their velocity and increase their time on both feet (double support) as a compensatory measure to improve stability. There was no difference in Base of Support despite commonly reported 'wide-based gait' in ataxic children. The GAITRite is sensitive to identify and quantify subtle abnormalities not seen clinically. Gait parameters which are statistically significantly different in the NGD cohort also correlates with mSST domains of Ataxia/Gait, Pyramidal and ExtraPyramidal. The mSST has the added advantage of accounting for the other neurological manifestations.

THE FREQUENCY OF THE N370S MUTATION IN THE GREEK POPULATION

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Introduction

The N370S (c.1226 G>A) mutation is exclusively associated with type I Gaucher disease (GD) and has a prevalence of more than 70% in Ashkenazi and up to 63% in non-Ashkenazi patients. However, studies in the Ashkenazi and Portuguese populations have shown that estimates of disease and gene frequencies, based on the number of diagnosed cases, are not reliable and present an underestimate of the true situation. In Greece, 82 type I GD patients have been diagnosed. In all but one, the N370S mutation has been detected either in homo- or heterozygosity accounting for 97/162 of the studied alleles.

Aim

The investigation of the true frequency of the N370S mutation by studying the genomic DNA purified from 1933 Guthrie cards, randomly sampled from the Greek National Neonatal Screening Program.

Method

Genomic DNA was isolated by boiling in 5% Chelex 100 resin and N370S was detected using mismatched PCR and digestion with the restriction enzyme XhoI.

Results

Eighteen heterozygotes and no homozygotes for the N370S mutation were identified. Thus, according to this finding, frequency of the N370S allele in the Greek population is 0.0046 with 95% confidence limits between 0.0025 and 0.0068. Applying the Hardy Weinberg equation, the expected number of homozygotes in our population of 11×10^6 individuals should be 238, i.e. 1:50,000 individuals should be a type I GD patient bearing the N370S/N370S genotype. However, in the last 25 years only 18 such cases have been diagnosed in our laboratory at the Institute of Child Health, which is the reference laboratory for Greece, and homozygosity through parental DNA analysis was confirmed in 5/18 patients.

Discussion

It has been proposed that only one third of N370S homozygotes are patients with GD. Should this be the case for our population too, 79 such patients should still have been diagnosed in Greece.

Our results clearly indicate a considerable underdiagnosis of N370S homozygotes and an underestimation of the frequency of the N370S in our population.

Acknowledgements

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**MANAGEMENT OF PATIENT WITH NON-NEUROPATHIC GAUCHER DISEASE
DIAGNOSE IN PREGNANCY**

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We describe the clinical course and management of a 35 year old patient with non-neuropathic Gaucher disease diagnosed in 2nd trimester pregnancy. The patient had 2 previous uneventful pregnancies at the age of 20 and 25 years followed by a miscarriage at the age of 33. At the age of 34 years she delivered a newborn with hydrops fetalis, who died shortly after birth. Hematological investigations were initiated because of increasing pancytopenia and splenomegaly during current pregnancy. Bone marrow histology showed lipid-laden cells suggestive of lysosomal storage disease. Genetic analysis revealed a compound heterozygosity (R131C/N370S) of the glucocerebrosidase gene. On referral to our department the patient displayed massive hepatosplenomegaly (liver and spleen size 156x184 mm and 300x87 mm, respectively). Laboratory investigations showed pancytopenia (leukocytes 3.6x1000/ μ l, hemoglobin 8.1 g/dl, platelets 30x1000/ μ l), glucocerebrosidase activity was 0.43 nmol/mg/min, chitotriosidase activity was 43,599 nmol/ml/h. MRI and echocardiography did not reveal any osseous manifestation or signs of pulmonary hypertension.

Enzyme replacement therapy (ERT) was initiated at 25 weeks of gestational age with imiglucerase, 60 U/kg weekly. After 10 weeks ERT chitotriosidase activity had fallen to 10,252 nmol/ml/h and pancytopenia had significantly improved (leukocytes 5.6x1000/ μ l, hemoglobin 9.8 g/dl, platelets 71x1000/ μ l). At 36 weeks she delivered a healthy male newborn, weighing 2,480 g, by elective cesarian section. Hysterectomy was carried out after cesarian section because of placenta increta with associated hemorrhage. Three days post partum the patient underwent laparotomy because of hemorrhage at the vaginal angle. She was discharged 11 days after delivery in good health.

Post partum, therapy with imiglucerase was reduced to 60 U/kg biweekly. At follow up 10 weeks post partum, the patient was in a good clinical condition. Hemoglobin was stable (10.9 g/l), chitotriosidase activity had decreased to 7,481 nmol/ml/h. Hepatosplenomegaly had improved (liver and spleen size 155x142 mm and 197x68 mm, respectively).

Thrombocytopenia shortly deteriorated on reducing ERT (37x1000/ μ l), but increased to 82x1000/ μ l during long term follow-up.

In conclusion, weekly imiglucerase therapy (60 U/kg) rapidly improved pancytopenia and hepatosplenomegaly in a patient with advanced Gaucher disease, permitting successful pregnancy and delivery.

P16

ACHIEVEMENT OF LONG-TERM THERAPEUTIC GOALS FOR ENZYME REPLACEMENT THERAPY IN PATIENTS WITH GAUCHER DISEASE TYPE I RECEIVING VELAGLUCERASE ALFA

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Introduction

Therapeutic goals have been described to monitor achievement, maintenance and continuity of therapeutic response in patients with type 1 Gaucher disease receiving ERT¹.

Aim

To benchmark the impact of velaglucerase alfa^a treatment against therapeutic goals¹ for 5 key clinical parameters of type 1 Gaucher disease (anemia, thrombocytopenia, hepatomegaly, splenomegaly and skeletal pathology).

Methods

In an open-label Phase I/II study, adults with symptomatic type 1 Gaucher disease and intact spleens received velaglucerase alfa for 9 months (60U/kg infusion every other week [EOW]). Eleven patients completed the study and 10 enrolled in a long-term extension. After 1 year, patients achieving ≥ 2 therapeutic goals began step-wise dose reduction from 60 to 45 then 30U/kg EOW. Data for anemia, thrombocytopenia, hepatomegaly, splenomegaly and skeletal pathology at baseline and 4 years are available for 8 patients (3 male, 5 female). The proportion of patients at goal for anemia, thrombocytopenia, hepatomegaly and splenomegaly at baseline was compared with the proportion achieving each goal at 4 years. The proportion achieving the skeletal pathology goal was determined on the basis of Z-score improvement from baseline to 4 years. The proportion of patients who achieved all 5 goals at 4 years was compared with the proportion at goal for all 5 parameters at baseline.

Results

At baseline, no patient was at goal for all clinical parameters (Table). After 1 year of treatment, all patients maintained goals present at baseline, and all achieved ≥ 2 goals. All 8 patients began step-wise dose reduction from 60 to 30U/kg EOW between 15 and 18 months. By year 4 of treatment, all patients met goal for all 5 clinical parameters; therefore 100% achievement was seen for each of the 5 long-term, therapeutic goals (Table).

	Baseline	Year 4
Anemia	4/8(50%)	8/8(100%)
Thrombocytopenia	0/8(0%)	8/8(100%)
Hepatomegaly	4/8(50%)	8/8(100%)
Splenomegaly	0/8(0%)	8/8(100%)
Skeletal pathology	—	8/8(100%)
All 5 goals	0/8(0%)	8/8(100%)

Discussion

In this velaglucerase alfa Phase I/II and extension study, clinically meaningful achievement of each long-term, therapeutic goal was observed for each patient, despite dose reduction after 1 year. This is the first report of a cohort where all patients receiving ERT for type 1 Gaucher disease achieved all 5 of these long-term, therapeutic goals within 4 years of starting treatment.

^aVelaglucerase alfa is approved in the US and is investigational in the EU

Pastores G, Weinreb N, Aerts H, Andria G, Cox T, Giralt M, Grabowski G, Mistry P, Tylki-Symanska A (2004) Therapeutic goals in the treatment of Gaucher disease; *Seminars in Hematology*, 41 (suppl 5): 4–14

INCIDENCE OF GBA MUTATIONS IN PARKINSONS DISEASE PATIENTS IN ARAGON

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A clinical association has been reported between Gaucher's disease, which is caused by a glucocerebrosidase deficiency owing to mutations in the glucocerebrosidase gene (GBA), and Parkinson Disease (PD). The aim of this study was to ascertain the frequency of GBA mutations in a cohort of Spanish PD patients and healthy age-matched controls. We screened a total 112 PD patients and 109 controls from Aragon, Spain, for GBA mutations by complete sequencing of all exons, as well as, intron/exon boundaries, of the GBA gene. We have found 7 patients (6,3%) heterozygous for GBA mutations (two L444P, one E326K, and four T369M) whereas only 4 control subjects (3,4%) carried GBA mutations (2 were heterozygous for N370S and 2 were heterozygous for T369M). The incidence of N370S mutation in control group was similar to the general Aragon population. By contrast, we have found a higher heterozygote frequency for the mutations in GBA gene in patients with PD compared with control subjects. Patients with PD had greater odds of being carriers of Gaucher's disease than did control subjects (odds ratio, 1,72; 95% CI, 0,51 - 5,77). Patients with PD carrying GBA mutations were younger than those not carriers (age at onset: 52,6±12,77 years vs. 60,2±10,20 years; p=0,065). In the present study, the clinical characteristics of patients with PD with GBA mutations (n = 7) were compared to those of patients with PD without any known GBA mutation. The overall clinical manifestations did not differ in patients with GBA mutations compared to patients without mutations. This study suggests that GBA is a susceptibility gene for PD and GBA mutations are associated with earlier age at onset of disease in this cohort.

PROINFLAMMATORY BONE CYTOKINE PROFILE IN GAUCHER DISEASE

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Bone disease (BD) is the most debilitating and disabling complication of Gaucher disease (GD), leading to bone marrow infiltration by Gaucher cells, failure of remodelling, osteopenia, osteonecrosis, osteosclerosis, bone crisis and chronic bone pain including pathological fractures. Gaucher cells activate and induce synthesis of proinflammatory cytokines that can modify the activity of osteoblast-osteoclast system and promote lytic phenomena. The aim of this study has been to identify the cytokine profile in GD1 patients with or without bone disease and to compare the results in a subgroup of homozygous N370S.

Two panels of cytokines including: A) IL2, IL4, IL13, IL17, EGF, Fractalkine, INF γ , MCP1, MIP1 β , TGF α ; B) IL4, IL6, IL7, IL10, IL13, MIP1 α , MIP1 β and TNF α were analysed in plasma samples by Luminex®100 platform and Millipore cytokine kits. We have analyzed two groups of GD1 patients at diagnosis: 16 heterozygous (females 50%, mean age: 42 year; range: 14-83) and 20 homozygous for N370S (females 60%; mean age: 43 years; range 23-63) and a group of 18 healthy controls (females 63%; mean age: 45 years; range 23-63). Kolmogorov-Smirnov analysis and mean comparative non parametric U-test was performed. In Panel A a significant high plasma IL-2 ($p=0.03$), MIP1 α ($p=0.001$), INF γ ($p=0.03$) concentrations in GD1 patients vs controls were observed. Moreover in Panel B GD1 homozygous N370S vs controls showed an increased plasma levels of IL-4 ($p=0.001$), IL-6 ($p=0.001$), IL-7 ($p=0.01$), IL-10 ($p=0.001$), MIP-1 α ($p=0.02$), TNF α ($p=0.001$). In addition we have detected a significant increase of IL13 ($p=0.02$) in GD1 patients homozygous N370S without bone disease. No significant differences in cytokine profiles related age and gender had been observed. In our experience Luminex®100 technology is a sensible and accurate technique useful to determine cytokine profile in plasma. A different significant cytokine profile was observed between GD1 patients and controls as well as between GD1 patients with and without bone affection.

CLINICAL; GENERIC CHARACTERISTICS AND GEOGRAPHICAL DISTRIBUTION OF GAUCHER DISEASE IN IBERIAN PENINSULAE

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The population of Iberian Peninsula (IP) is about 55 millions inhabitants with different ethnic groups. The aim of this study is to describe the distribution and clinical characteristics of GD patients in IP. Data from the National Spanish Gaucher Disease Registry (since 1993), Bioquímica Clínica Institut-Genetic Department of Barcelona University (since 1970) and the Portuguese Coordinating Committee for the Treatment of Lysosomal Storage Diseases (1993-2005) were jointed. Statistical analysis of demographic data, geographical distribution of GBA mutations and allelic frequency of mutations were analyzed. As well as, to identify the existence of clusters and the distribution of different types, the age at diagnosis (aDx), SSI, type of therapies and years on. The prevalence had been 436 GD patients, (421 born in IP). Mean aDx: 26.3±19.88 y. Females: 47.74 %, males: 47.98%, unknown: 4.28%; type 1: 375 (89.1%), type2: 27(6.4%), type3: 19 (4.5%). Mean SSI in type1 GD: 7.7 (range 1-30), 72.7% mild, 25.5% moderate and 1.7% severe. We have identified 70 alleles and 76 different mutations. The most prevalent allelic frequencies: N370S: 48.8%, L444P: 18.3%, D409H: 3.1%, G377S: 2.9%, double mutation: L444P+E326K: 2.0%, which account the 75% of total alleles, in 3.3% of alleles the mutation has not been yet identified and the remaining alleles corresponding to private mutations. The most frequent genotypes were N370S/L444P, 27.8%, N370S/N370S, 15%, N370S/? 5.2%, G377S/D409H, N370S/G202R, N370S/c.84insG, 2.4% respectively. N370S is more frequent in Portugal vs Spain (57.1% vs 46.5%) and L444P is more frequent in Spain vs Portugal (19.4% vs 14.3%). 247 patients (58.7%) have received ERT (mean: 10.2±3.89 y); 53 (12.6%) has been treated with miglustat (mean: 2.1±1.9 y). The 90.4% of type1 and 31.0% of type 3 are alive and all type 2 are dead. In conclusions there are broad spectrums of GBA mutations in GD patients from IP. Five different mutations account for 75% of GBA mutant alleles. No differences in gender distribution and tendency of aDx were observed. The 98.2% of type1 GD has a mild or moderate grade of severity and 23.0% has never received ERT or SRT.

SAFETY WITH VELAGLUCERASE IN TWO GIRLS PREVIOUSLY TREATED WITH IMIGLUCERASE

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The incidence of immunologic reactions in patients with Gaucher Disease on enzymatic replacement therapy (ERT) with Imiglucerase is 17% and related with the presence of non neutralizing IgG antibodies. Clinical trials with Velaglucerase alpha have demonstrated lower immune reactions and very low antibodies against the enzyme even in patients that had previously developed antibodies against Imiglucerase. We report two cases with severe adverse immune reactions during and after infusion of Imiglucerase due to presence of antibodies. Case 1: female, 13 yo. Genotype N370S/84GG. She started ERT with Imiglucerase (60 U/kg eow) at 4 yo. After the fourth infusion she developed generalized skin rash with respiratory difficulties, headache and fever during infusion and 6-8 hours after; controlled with steroids and antihistaminic. She required after this event both low infusion rate (6 hours or more) and premedication with antihistaminic, dyfendramine and rest at home for 24 hours. IgG antibodies against Imiglucerase were positive. Therapeutic goals were satisfactory after twelve months on ERT, with normalization of blood counts, reduced spleen volume and growth recovery. After 8 years under Imiglucerase the patient was switched to Velaglucerase (compassionate use) at identical dose 33U/kg (1.200 U/eow) IV during 1 hour without any premedication. No adverse event has appeared and the clinical and analytical parameters have remained stable. No Velaglucerase antibodies have been detected after 12 months on therapy. Case 2: girl 4 yo. Genotype N370S/IVSA4-2^a>G; c(-203)A>G. She started Imiglucerase (60 U/kg eow), and three months later developed generalized skin rash with respiratory difficulties and vomiting during three consecutive infusions and required treatment to be stop. Imiglucerase IgG antibodies were positive. In September 2009 the patient started Velaglucerase (compassionate use) at 60U/kg (1.200 U/eow) IV during 1 hour without premedication. At present, the tolerance is good, no palpable spleen enlargement has been detected, blood counts and biomarkers are normal and no Velaglucerase antibodies have been detected.

UPDATED RECOMMENDATIONS FOR THE MANAGEMENT OF PREGNANCY IN GAUCHER DISEASE

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Introduction

Pregnancy in Gaucher disease (GD) may exacerbate anemia, thrombocytopenia, organomegaly and bone disease compounding risk of complications in pregnancy, delivery and post partum. EMEA guidelines indicate that treatment-naive women should be advised to consider commencing imiglucerase before conception to obtain optimal health and, in women already receiving imiglucerase therapy, continuation through pregnancy should be considered. Questions remain, however, on indications for treatment, and the optimal management of GD around pregnancy.

Aim

To determine best practice in the management of pregnancy in GD and prepare recommendations that may help physicians achieve uncomplicated, successful outcomes.

Methods

Authors, with seven other international experts in GD management, previously performed a comprehensive survey of outcomes of pregnancy management in GD¹. Based on this survey of 78 alglucerase/imiglucerase-treated and 338-untreated pregnancies in author's treatment centres; 42-treated and 356-untreated pregnancies in the literature; 114 pregnancies in a Canadian survey, and the authors' own clinical experiences, recommendations for the optimal management of pregnancy were agreed.

Results

There is no need to initiate imiglucerase therapy in asymptomatic females because they wish to, or become, pregnant. For untreated patients or those diagnosed during pregnancy, imiglucerase may be considered in symptomatic patients e.g. platelets $80-100 \times 10^9/L$ and/or evidence of deteriorating bone status. If a patient has a low platelet count or bleeding tendency, it is recommended that imiglucerase is initiated to avoid possible bleeding complications. All women with GD may be at risk of postpartum bleeding¹ and should deliver in medical centers where immediate access to blood transfusions is available. Platelet function tests are required to prepare replacement blood products and help determine mode of anesthesia. There is no specific GD indication for caesarean delivery. For uncomplicated vaginal deliveries, mothers may be treated as non-GD mothers and monitored as inpatients according to local policies. After operative delivery, monitoring of GD mothers as inpatients for 24-48 hours is advised due to an increased risk of bleeding complications. If a mother received imiglucerase during pregnancy, she may be encouraged to continue through breast feeding and can be reassured that there is no evidence of risk for the baby.

Discussion

With no evidence of adverse effects on the fetus or infants breastfed by mothers receiving imiglucerase, physicians can be reassured in treating women during pregnancy and lactation. If GD is controlled, women are more likely to experience uncomplicated pregnancies and deliveries.

Zimran. et al. Blood Cells Mol Dis. 2009;43(3): 264-88.

GENOTYPING PITFALLS IN A FAMILY WITH GAUCHER DISEASE

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Gaucher disease (GD) is an autosomal recessive disorder caused by mutations in the lysosomal glucocerebrosidase (GBA) gene. The diagnosis of GD is based on the determination of glucocerebrosidase activity. Usually to identify GBA mutations by sequencing, the entire structural gene is amplified by long PCR in order to avoid the amplification of pseudogene, followed by nested PCR and direct sequencing. Alternatively, restriction enzyme digestion of previous PCR fragments is used for analysis of N370S and L444P mutations. Here we present a 3-years old boy diagnosed as GD (genotype: [N370S]+[c.(-203)A>G;P182L]) whose mother was classified as homozygous for N370S mutation by direct sequencing, meanwhile she was diagnosed as heterozygous for N370S by restriction isotyping. The same pattern was observed for her sister in the analysis of complex allele with the c.(-203)A>G and P182L variants. We hypothesized that these pitfalls must be due to a mutation that prevents the amplification of one of the mother alleles by long PCR which is inherited by the daughter. We performed alternative PCR assays with primers which anneal to GBA gene and not to pseudogene, direct sequencing of these fragments confirms results obtained with restriction analysis, and designed other primers to amplify the surroundings of the region in which primers for long PCR anneal to look for the cause of our results. In conclusion, we describe a potential pitfall in DNA sequencing, indicating that in specific cases is not possible to perform a correct genetic GD diagnosis by direct sequencing of the GBA gene.

RegistryNXT!: NEW TECHNOLOGY TO ENHANCE DATA COLLECTION AND REPORTING FOR THE LYSOSOMAL STORAGE DISORDER REGISTRIES

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Introduction

The Lysosomal Storage Disorder (LSD) Registries program, initiated in 1991, includes international disease registries for Gaucher, Fabry, MPS I, and Pompe disease, and is supported by Genzyme Corporation. For rare diseases such as the LSDs, a full appreciation of the world-wide spectrum of the disease is only possible through longitudinal international disease registries with large populations of patients. The designs of these registries involve scientific and technological challenges which include the collection of longitudinal, voluntary, retrospective data. The systems for data collection are critical to the success of each registry.

Over the last ten years, these LSD Registries have successfully transitioned from paper case report forms to electronic data collection. Now, a multi-year, multi-registry technology and design initiative, known as RegistryNXT!, is underway.

Aim

To report on RegistryNXT!, the redesigned data collection technologies and data reporting systems that underlie the LSD Registries.

Methods

RegistryNXT! completed a multi-registry planning phase and the reporting requirements and system design for the Gaucher Registry. A build-phase is underway and a launch of the Gaucher Registry's new RegistryNXT! system is planned for late 2010.

Results and Discussion

These efforts have identified key needs for Registry participants, including more immediate access to patient data and more flexible, easy-to-use reporting tools. New features, such as interactive patient case reports, will enable near real-time disease management support or the option to share reports with other members of the healthcare team or with patients. The RegistryNXT! system will also support more rapid analyses of aggregate Registry data for research. A prototype of the RegistryNXT! system will be demonstrated showing some of the versatile features that will maintain these Registries as the leaders in data collection which has helped to increase the awareness and improve the knowledge of these rare diseases.

**DYSGAMMALGLOBULINEMIA IN GAUCHER DISEASE:
DATA FROM 61 PATIENTS; INCLUDING 18 PATIENTS WITH GD TYPE III**

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Aims

We performed a study to assess the prevalence of dysgammaglobulinemia in patients with Gaucher disease (GD) that were known from the outpatient clinic for inherited metabolic disorders at the Children's Memorial Health Institute (CMHI), Warsaw, Poland.

Methods

Total serum concentrations of IgG, IgA and IgM were determined by nephelometry. The presence of monoclonal proteins was determined by immunoelectrophoresis. To confirm and characterize monoclonal proteins, immunofixation was performed.

Results

Sixty one patients with GD (43 with type I and 18 with type III) were evaluated for the presence of gammopathies. The median age was 29.66 (range, 0.66 –77.33), 25 (41%) were male and 36 (59%) were female. 41 patients (67%) had normal levels of immunoglobulins, while 20 (33%) showed presence of dysgammaglobulinemia. Hypogammaglobulinemia was observed in 5 patients (1 IgG; 4 IgM), while 15 had hypergammaglobulinemia (5 IgG; 6 IgA; 3 IgM; 1 IgG + IgM). Among 20 patients with dysgammaglobulinemia, 5 were splenectomized, 15 were treated with enzyme replacement therapy (ERT) with imiglucerase (median 9 yrs, range 0.4 - 15), 1 with miglustat and 4 were untreated. Only 2 patients (3.3% of the study population) had monoclonal gammopathy (MG), (1 IgG λ ; 1 λ). Both of these patients had type I GD, one was 78 years old and untreated and the other was 65 years old and treated with ERT for 5 years, neither was splenectomized.

Discussion

Our study population shows relatively low prevalence of MG (3.3 %) among patients with GD, despite the fact that approximately 30% of patients had GD type III. The development of MG increases with age and both our patients with MG were relatively old and either untreated or started therapy after the age of 60 years. Majority of the patients have been receiving long-term ERT and their disease status was very stable. Similar to other studies, we conclude that long term ERT has positive effect on reducing MG in GD patients.

Ph-POSITIVE CHRONIC MYELOID LEUKEMIA IN PATIENT WITH GAUCHER DISEASE (Clinical vignette)

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Introduction

Gaucher patients having abnormal metabolism also can suffer from other illnesses, that should be treated without interrupting enzyme replacement therapy. Here we report a case of such combination successfully treated.

Case Report

The female patient was born in 1946. Gaucher disease was diagnosed at the age of 27 years (in 1973). The patient did not receive ERT and had no complaints up to year 2003. Since 2003 the patient has been feeling bad. In December 2004 the patient was admitted to the National Research Center for Hematology in severe condition: weight 48 kg (height 158 cm), significant signs of anemia (60 g/l), hemorrhages on skin, giant spleen occupying nearly the whole abdomen cavity. Since December 2004 the patient receives ERT. On this treatment the patient's condition improved significantly, Hemoglobin (Hb) and Platelets levels increased. Since May, 2006 – deterioration: fatigue, enlargement of spleen, neutrophilic leukocytosis with the shift to the left. Bone marrow puncture and biopsy revealed myeloid metaplasia and Ph-chromosome found in 92% of the cells. Thus, Ph-positive chronic myeloid leukemia (chronic phase) in the patient with Gaucher disease was diagnosed. The treatment with imatinib was started again. The patient was still receiving ERT (30 U/kg). Complete hematological remission was observed after 6 months of treatment (Hb 120 g/l, Platelets 60,0, Leukocytes $5,0 \times 10^9 / \text{mm}^3$). However, there were still 3% of Ph-positive cells. During the next 6 months the patient has not received treatment with imatinib (till November, 2007). She was feeling well and received 15-30 U/kg/infusion of ERT regularly. The spleen reduced significantly: +1-2 cm below costal margin. However, the control bone marrow puncture revealed 60% Ph+ cells, so the treatment with imatinib was started again (in December 2007).

Discussion

At present time we observe hematological and molecular remission of myeloid leukemia (0,04% BCR-ABL cells), imatinib therapy continues. Gaucher disease: the goals of treatment are achieved, no spleno- and hepatomegaly can be seen. Hb 120g/L, Pl. – $70 \times 10^9 / \text{L}$, L. – $4,0 \times 10^9 / \text{L}$.

Patient continue receiving ERT in dose 800U/infusion biweekly. We are keeping the patient under observation.

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BONE DENSITY CHANGES IN CHILDREN WITH GAUCHER DISEASE **A proposal for an international collaborative effort**

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Introduction

Bone involvement is perhaps the most variable symptom of Gaucher disease and a major cause of morbidity. The exact incidence of skeletal complications is not known but an early estimate of approximately 80% has been suggested. Interestingly, there is not necessarily a correlation between severity of bone involvement and severity of any other parameters of Gaucher disease so that skeletal involvement may be the primary symptom.

Dual energy x-ray absorptimetry (DEXA) at the femoral neck (FN) is the 'gold standard' for the assessment of osteoporosis and has been shown to be a sensitive and specific indicator of skeletal involvement in Gaucher disease. The advantages of DEXA include low radiation dose, high precision, and availability; attributes especially relevant in Gaucher disease since long-term surveillance is required.

Aim

Measurement of Bone Mineral Density (BMD) status would be a useful tool in identifying the children with Gaucher disease who could be exposed to an increased risk of osteoporosis in adulthood. Indeed in a recent international forum one of the key areas for research development in Gaucher disease included the requirement for standards for monitoring of bone disease particularly in infants and children.

The use and correct interpretation of BMD measurements in pediatric patients relies on the availability of appropriate reference data. Ideally, such data should be matched for sex, chronological age, height, weight, pubertal development, and ethnicity. Recently, such standards have become available for the Hologic system, and hence a longitudinal study of BMD at the FN and lumbar spine of children with Gaucher disease is proposed.

Study design

To perform DEXA examinations for patients aged 5-25 years every 12 months, regardless of severity of Gaucher disease for 2 years. Vitamin D and calcium levels as well as bone metabolite markers will be taken at these intervals and wrist x-rays for bone age will be used to correlate bone and chronological age. Approval of IRB is needed. Parents consent for this exam will be needed also since this modality has been part of long term follow-up in adults but not in children and adolescents. Data will be evaluated in comparison to normative values for children, and in relation to duration of ERT, and other evaluators of disease severity.

CLINICAL DATA OF SPLENECTOMIZED AND NON-SPLENECTOMIZED GAUCHER PATIENTS IN RUSSIA

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Introduction

In the last 5 years the number of Gaucher patients in Russia increased more than five times and now exceeds 250.

Aim

Characterize clinical and laboratory parameters in treatment-naïve adult patients with Gaucher disease type 1. We also compared our data with the data from ICGG Gaucher Registry.

Methods

The group included 71 patients aged from 16 to 75 years (mean age 34 yrs) – 24 males (mean age 30 yrs) and 47 females (mean age 35,5 yrs). Gaucher disease was diagnosed in 36 (51%) patients elder than 18 years, and in 35 (49%) patients in childhood. The diagnosis was based on bone marrow examination and/or histological examination of spleen (in 41% of patients) and in all cases was confirmed by enzyme assay. In 25 patients splenectomy was performed in childhood, in 4 patients – after the age of 18 years (one of them had traumatic rupture of spleen).

Results

The leading clinical signs in non-splenectomized patients were significant splenomegaly (mean size +12 cm below costal margin) and profound thrombocytopenia (mean platelet count $50,0 \times 10^{12}/L$). Normochromic or hypochromic anemia was found in 23 (55%) patients, in 7 (30%) of them anemia was moderate (<90 g/L). Hepatomegaly without liver dysfunction was observed in 36 (86%) patients. Severe bone involvement (pathological fractures, avascular necroses of femur heads with severe arthroses) was revealed in 6 (14%) patients.

The leading clinical sign in splenectomized patients (n=29) was hepatomegaly (mean size +8 cm below costal margin). Platelet count was normal in all patients except 2 (7%) individuals. Anemia was found in 12 (41%) patients, mild (90-110 g/L) in absolute majority of patients. Pathological fractures and avascular necroses of femur heads were revealed in 14 (48%) splenectomized patients.

Discussion

Demographic and clinical data of treatment-naïve adult Gaucher patients in Russia are comparable with the corresponding data from ICGG Gaucher Registry. For May 2010, 90% of 71 Gaucher patients receive treatment: most of them (84%) receive ERT, 16% – new treatment with small molecule – eliglustat tartrate.

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF) NOVEL MUTANT ALLELES CAUSING GAUCHER DISEASE

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Gaucher disease (GD) is caused by the defective activity of the acid lysosomal β -glucosidase (GBA), resulting in the accumulation of sphingolipids within the lysosomes. GBA is transported to the lysosomes in a mannose-6-phosphate independent manner through its interaction with the lysosomal integral membrane protein type 2 (LIMP-2). It has been shown that the trafficking to the lysosome of certain GBA mutant proteins that are retained in the ER may be rescued by the over-expression of LIMP2. However, the impact of GBA mutations on the interaction between GBA- LIMP-2 has not been explored in detail.

We report here the identification and functional characterization of 8 novel GBA mutant alleles, including 7 missense mutations P159S, N188I, P245T, W312S, S366R, W381C, L385P and one complex allele, N188S+G265R. Mutations P245T, S366R, W381C and L385P were found in 4 GD1 patients, as compound heterozygote with N370S, while W312S was present in a GD1 patient in association with the L444P mutation. The allele N188I was found in a GD2 patient in association with mutation R131C, while mutation L159S and the complex allele N188S+G265R were present in two different GD3 patient in heterozygosis with mutation E326K and an uncharacterized mutation, respectively. Mutant proteins were expressed in vitro in Hek293 cells and assayed for enzymatic activity and protein processing. All mutant proteins were inactive except for the P159S that presented a 13% of wild type activity. To further characterized the N188S+G265R allele, we expressed in vitro constructs bearing the single mutations (N188S and G265R). While G265R mutant expressed no activity, the I188S expressed an activity of 25 % with respect to wild type activity, as previously reported. It is worth of note that the enzymatic activity of the proteins bearing mutations at position 188 (N188S and N188I) was completely different: the change of Asn with Ser (both polar aminoacids) leads to the synthesis of an enzyme that retains some residual activity, the change of Asn with Ile (a non polar aminoacid) completely abolishes the enzymatic activity. In addition, to analyze whether these mutations affect the binding between GBA and LIMP-2, Hek293 cells were transfected with wild type and mutant constructs and the GBA-LIMP2 interaction was analyzed by co-immunoprecipitation. The results showed a different impact of GBA mutations on GBA binding to LIMP2, providing new insights into the nature of GBA-LIMP2 interaction.

VELAGLUCERASE AS ENZYME REPLACEMENT THERAPY IN TYPE 1 GAUCHER DISEASE: REPORTING THE FIRST UK NON-TRIAL PATIENT

Milligan A., Baker R., Cooke J., Hughes D.A., Richfield L. B., Steel L. and Mehta A.B.

Lysosomal Storage Disorders Unit, Royal Free Hospital, London. 2010.

Background

Global shortages of Cerezyme® in 2009 presented numerous and complex treatment challenges for patients and clinicians managing their care. The majority of patients had reduced dose/frequency, switched to SRT or taken a total treatment break depending on individual circumstances. We present a brief case history of a 19 year old female with a recent diagnosis of type 1 Gaucher referred to our centre in August 2009 when licensed enzyme replacement was in very short supply.

History

Despite a symptomatic history of severe bone and joint pain from the age of 4 yrs, clinical suspicion of Gaucher was not raised until she was referred at the age of 19 to a haematologist at her local hospital with a pancytopenia. A bone marrow aspirate was performed to exclude haematological malignancy. Results revealed diffuse infiltration of Gaucher cells. Disease was confirmed enzymatically and referral made to our centre for ongoing management. Mutational analysis identified a rare genotype likely to be associated with a severe phenotype (c.667t>c p.W184R) homozygote. Medical assessment confirmed progressive disease with significant organomegaly and skeletal involvement evident on MRI.

Initiation of ERT

Shire® allowed early access to unlicensed Velaglucerase so as to initiate ERT without further treatment delay. Our internal, Hospital Drugs and Therapeutics Committee approved prescribing of Velaglucerase on a named patient basis. In October 2009 the subject became the first patient in the UK to start ERT with Velaglucerase (outside a clinical trial) on the NHS. First infusions given in the hospital were tolerated without event. She is studying at University and now continues to receive home infusions with a home nursing visit to her student accommodation 2 weekly. Test results at 4 month treatment follow up showed an increase in Hb. from 11.4 g/dL to 12.2 g/dL, increased platelet count from 78 to 90 x 10⁹/l. and a reduction in splenic volume on abdominal examination.

Conclusion

It is unfortunate that an accurate diagnosis was not made earlier in life at a time when ERT with Ceredase® was available to children with early manifestations. Aged 7, a 'supposed' diagnosis of osteomyelitis had been made in this patient, although no positive cultures were ever obtained. A Hickman line was placed through which IV antibiotic therapy was administered for 6 months. In the UK the NCG (National Commissioning Group) system of designated specialist centres has proved effective in developing a collaborative and strategic approach to the ongoing global shortages of licensed ERT.

PLATELET FUNCTION AND COAGULATION ABNORMALITIES IN TYPE 1 GAUCHER PATIENTS: EFFECTS OF CEREZYME® THERAPY

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Introduction

Coagulation abnormalities and platelet dysfunction were reported in Gaucher patients (GP). Data concerning the effects of enzyme replacement therapy (ERT, Cerezyme®) on coagulation abnormalities and platelet function are few and controversial.

Aim

To investigate the impact of Cerezyme® on haemostatic parameters of GP.

Methods

31 Serbian treatment-naive type 1 GP (M/F 17/14; median age 49 years, splenectomized 9/31) were studied. Complete blood count, prothrombin time (PT), activated partial thromboplastin time (aPTT) and coagulation factors activities were measured according to standard methods. Platelet aggregation (PA) was assessed by a whole-blood aggregometer with collagen (Col) 10 and 5µmol/L, adenosine5-diphosphate (ADP) 10 and 5µmol/L, ristocetin (Ris) 1.25mg/ml, epinephrine (EPI) 10µmol/L and arachnoid acid (AA) 2mmol/L (normal PA≥60%). Spleen volumes (SV) were assessed by CT. 21/31 patients were treated with Cerezyme®. Haemostatic parameters were assessed after 6, 12 and 24 months (ERT_{6, 12, 24})

Results – Pretreatment

Bleeding episodes were registered in 10/31GP. Mean platelet count (PC) was 150x10⁹/L; 22/31 GP were thrombocytopenic. PT (median 61%) and APTT (median 41.45s) values were abnormal in 16/31 and 13/31 GP respectively. PA abnormalities were registered in 19/31 GP. Mean PA was reduced in response to EPI (0.57) and AA (0.58). SV inversely correlated with PC and reduced response to Col₅, AA and ADP₅ (p<0.05). Bleeding GP had significantly lower PC, higher chitotriosidase level and greater SV comparing to the nobleeding (p<0.01).

After ERT

N^o of bleeding GP significantly decreased after ERT₆ (1/10 GP; p<0.01) and disappeared at ERT₂₄. PC significantly increased at ERT₆ and remained in normal range till ERT₂₄ (Plt₆ 240x10⁹/L, Plt₁₂ 240x10⁹/L, Plt₂₄ 295x10⁹/L; p<0.01). PT increased significantly from ERT₀ to ERT₂₄ (PT₀ 75%, PT₆ 73%, PT₁₂ 77%, PT₂₄ 85%; p<0.01). vWF increased significantly at ERT₆ and ERT₂₄ (vWF₀ 65%, vWF₆ 68%, vWF₁₂ 65%, vWF₂₄ 87%; p<0.01). N^o of GP with abnormal PA decreased significantly at ERT₆ (5/19; p<0.05). PA on AA and EPI normalized at ERT₆: 0.76 and 0.72 respectively. Chitotriosidase level and SV significantly decreased achieving: Chito₂₄ 1872 nmol/L p<0.05, spleen MN₂₄ 9, p<0.05.

Discussion

Bleeding together with decreased levels of coagulation factors and abnormal PA were registered in a considerable number of GP. Cerezyme significantly decreased bleeding tendency and increased PC, PA and PT.

P31

GBA MUTATIONS AND PARKINSONS DISEASE

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Introduction

An association between Parkinson's disease and the presence of mutations in the GBA gene has been demonstrated by the concurrence of Parkinson's and Gaucher (GD), the identification of GBA mutations in probands with PD and neuropathological studies of patients diagnosed with GD, parkinsonism and dementia.

Aim

The investigation of the presence of mutations in the GBA gene in Greek patients with PD.

Patients – Methods

The study group consisted of 100 unrelated ethnic Greek patients with sporadic Parkinson's disease who visited Outpatient Clinic at University Hospital of Larissa (Thessaly, Central Greece) and 105 age- and sex- matched controls with no family history of PD, recruited through a previous epidemiological survey in Thessaly. All were tested with PCR followed by restriction enzyme analysis for the GBA mutations: N370S, D409H, L444P, R463C/IVS10-1G→A, R120W, Y108C, IVS7-2A→G, and H255Q which cover approximately 87% of the mutations of Greek GD patients. Statistical analysis was performed by the χ^2 test and t-test.

Results-Discussion

Our study identified 11 PD patients and 3 controls as carriers of mutations in the GBA gene. In the PD group N370S (4/11), L444P (2/11), H255Q (1/11) and D409H/H255Q (2/11) and in the controls N370S (1/3), H255Q (1/3) and Y108C (1/3) were identified. Thus, a significant association between PD and mutations in the GBA gene is found in the cohort of patients studied ($p=0.021$; OR 4.2; 95% CI=1.136-15.54). Furthermore, the age of onset of PD symptoms in patients that were carriers of GBA mutations was earlier than in non-carriers, the difference being statistically significant ($p=0.034$). In conclusion, our results show that in the cohort of patients studied and in agreement with previous reports, GBA gene mutations are a risk factor for Parkinson disease and their presence is associated with earlier onset of symptoms.

P32

WELLBEING OF MANCHESTER PATIENTS WHO CHANGED OVER FROM IMIGLUCERASE (CEREZYME) TO VELAGLUCERASE IN THE HOSPITAL AND THE HOME SETTINGS IN CHILDREN WITH TYPE I AND TYPE III GAUCHER DISEASE

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Introduction

Gaucher Disease is the most common Lysosomal Storage Disorder and the first for which enzyme replacement was used as a therapy. In July 2009 all our Gaucher patients had to have an enforced reduction of Cerezyme. Patients consisted of 11 Type I and 3 Type III children all who have received therapy for between 18 months and 18 years. The 3 Type III patients are all treated at home, whilst 2 Type I's are infused at their local hospital and the remainder treated by parents or Home care nurses in the home setting.

Aim

To investigate the wellbeing and efficacy of switching treatment from Cerezyme to Velaglucerase during the recent shortage of the former.

Results

The poster illustrates the wellbeing of 2 type III patients and 8 type I patients who opted to change from Cerezyme to Velaglucerase from November 2009 to February 2010. The remaining 4 patients continue on Cerezyme every 2 weeks. There were no adverse events during this period.

Discussion

Pre switch we monitored all patients by measuring Chitotriosidase on a monthly basis and we will continue to monitor the patients, both clinically and by measurement of biomarkers and haematological parameters, throughout the switch period.

HOME INFUSIONS PROVIDE A CONVENIENT OPTION FOR INTRAVENOUS ENZYME REPLACEMENT THERAPY WITH VELAGLUCERASE ALFA PATIENTS WITH TYPE I GAUCHER DISEASE

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Enzyme replacement therapy (ERT) is the standard of care for symptomatic patients with type 1 Gaucher disease. In Israel, patients have the opportunity to receive home therapy after undergoing the first 3 infusions at their medical center; hence, patients enrolling in clinical trials with emerging ERTs increasingly expect the same benefit. We were therefore encouraged by the innovative use of home therapy in the velaglucerase alfa clinical trial program since home therapy facilitates participation, especially in trials continuing into a long extension phase.

The experience of the infusion nurse is pivotal to the success of ERT administration in the home setting. It is the nurse's responsibility to collect the drug vials in a cold pack at the study site's Pharmacy and travel with them to the patient's home. The drug is re-constituted at the patient's side and infused immediately. Vital signs are recorded at intervals, and AEs and concurrent medications are updated in the nurse's report. The nurse remains at the patient's side until the infusion is completed and then is available for 60 minutes post-infusion to monitor vital signs.

Our center participated in all the velaglucerase alfa clinical trials: TKT025 (and extension), TKT032, TKT034 and HGT-GCB-039 (and their extension HGT-GCB-044) and, as is our typical practice, patients received infusions at home where possible (**table**). Per protocol, all patients had their first 3 infusions at the study site. Providing they had not experienced a serious or infusion-related AE, certain of the trial protocols then allowed that patients could receive home infusions from a qualified person, at the investigator's discretion. All patients were required to return to the study site periodically.

In conclusion, we believe that home therapy, where supported by experienced nurses, provides a convenient option for patients receiving enzyme infusions for Gaucher disease.

		Total patients enrolled & dosed (n)	Total patients who received home therapy (n)	Patients from Shaare Zedek site who received home therapy (n)
TKT025	Phase I/II first-in-human safety study in treatment-naïve patients	12	Not allowed per protocol	Not allowed per protocol
TKT025EXT	Long-term extension of TKT025	10	7 ^b	7 ^b
TKT032 ^a	Parallel dose study in treatment-naïve patients	25	Not allowed per protocol	Not allowed per protocol
TKT034 ^a	Switch study in patients previously receiving imiglucerase	40	25 ^b	9 ^b
HGT-GCB-039 ^a	Head-to-head comparison of velaglucerase alfa and imiglucerase in treatment-naïve patients	34	Not allowed per protocol	Not allowed per protocol
HGT-GCB-044 ^a	Long-term extension	95	35 ^b	15 ^b

a. Multicenter trials.

b. As of 31 August 2009.

Velaglucerase alfa is approved in the U.S; it is an investigational product in Europe.

COMPARISON OF IN VITRO CELLULAR UPTAKE OF VELAGLUCERASE ALFA TO THAT OF IMIGLUCERASE: Effects of Chemical Parameters and Receptor-Specific Inhibition

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Introduction

Velaglucerase alfa (Shire HGT), an acid- β -glucocerebrosidase for patients with type 1 Gaucher disease, binds to and is internalized by macrophages via the macrophage mannose receptor (MMR), to catalyze the degradation of accumulated intracellular glucocerebroside. The efficiency of cellular internalization may impact its efficacy and/or safety.

Aim

To develop an in vitro cellular uptake assay, and to use this assay to compare uptake of velaglucerase alfa to imiglucerase.

Methods

For Design of Experiments (DOE) assays, general factorial design was assisted by Statease Design Expert™ software. DOE utilized macrophages derived from phorbol myristate acetate (PMA) induced U937 cells, and were conducted in the presence of 5 mM mannose-6-phosphate (M6P). For internalization comparisons, U937-derived macrophages were incubated for 3 hours with GCB at pH 7.5 with 10 mM calcium. Internalized drug was measured by an activity assay with a synthetic substrate (4-MU-glc) that fluoresces upon cleavage.

Results

Comparison in U937 cells of the internalization rates of velaglucerase alfa and imiglucerase showed that velaglucerase alfa is internalized approximately 2.5 fold more efficiently than imiglucerase *in vitro*. This differentiation in cellular internalization was also observed using the MMR-expressing murine cell line J774. Under specific assay conditions, the addition of calcium mildly inhibited the cellular uptake of imiglucerase while it enhanced the uptake of velaglucerase alfa. The internalization of both enzymes could be inhibited by addition of mannan to the culture medium, although the inhibition of velaglucerase alfa uptake by J774 cells was more complete than that of imiglucerase. DOE assays revealed that: i) the interaction of calcium with pH greatly impacts uptake; and ii) bioassay sample comparisons required the presence of calcium, consistent with the known calcium-dependence of the MMR. The presence of mannose-6-phosphate (M6P) in DOE experiments ensured that the M6P receptor on U937 cells did not contribute to the measured internalization.

Discussion

These data suggest that velaglucerase alfa is internalized more efficiently than imiglucerase. While both enzymes are primarily internalized via the MMR, a small portion, greater for imiglucerase than for velaglucerase alfa, is internalized by an alternative mechanism. These data may prove valuable in differentiating velaglucerase alfa, imiglucerase, and other future therapies.

MRI BONE MARROW FINDINGS IN 63 PATIENTS WITH TYPE I GAUCHER DISEASE

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Introduction

Avascular necrosis (AVN) of the femoral head is probably the most disabling skeletal manifestation in Gaucher disease [1]. MRI is the method of choice for assessing bone marrow involvement in adult Gaucher patients [2].

Aim

To determine whether MR bone marrow findings in Gaucher patients may help to identify patients at high risk of developing severe Gaucher bone complications exemplified by avascular necrosis of the femoral head.

Methods

MR images were obtained in 63 Type I Gaucher patients through a standard protocol using coronal T1- and T2-weighted sequences of the lower extremities. The location and extent of infiltrated marrow was established using a semi-quantitative MRI scoring method (Düsseldorf Gaucher score, DGS) and the morphological pattern of bone marrow involvement determined (whether homogeneous type A or non-homogeneous type B). Active marrow process with bone oedema and AVN of the femoral head were also analysed.

Results

Bone marrow involvement was observed in femoral sites more than in tibial sites. A high DGS was significantly correlated with type B morphology and femoral AVN (both $p < 0.0001$). Splenectomised patients showed a significantly higher Düsseldorf Gaucher score and type B morphology than non-splenectomised (both $p < 0.05$). AVN was seen in 46% of patients with type B morphology versus 3% in type A morphology ($p < 0.0001$). DGS and morphology of bone marrow involvement were not significantly correlated with active marrow processes [3].

Discussion

Type B marrow morphology and extensive marrow packing were significantly associated with AVN of the femoral head (both $p < 0.0001$). These patterns are considered predictive and may be employed in a disease management context to alert physicians to the need for urgent therapeutic measures.

Weinreb N, Barranger J, Packman S et al. Imiglucerase (Cerezyme) improves quality of life in patients with skeletal manifestations of Gaucher disease.

Clin Genet 2007; 71: 576-588

1. Poll LW, Koch J-A, vom Dahl S, et al. Magnetic resonance imaging of bone marrow changes in Gaucher disease during enzyme replacement therapy: first German long-term results. Skeletal Radiol 2001; 29: 563-571
2. Poll LW, Willers R, Häussinger D, Mödler, vom Dahl S. MRI bone marrow findings in 63 patients with type I Gaucher disease. Fortschr Röntgenstr 2010, in press

IDENTIFICATION OF BIOMARKERS OF GAUCHER DISEASE USING PROTEOMIC TOOLS

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Several biomarkers show altered accumulation in the plasma of Gaucher Disease (GD) patients which can be used for diagnosis and follow-up. Chitotriosidase protein (ChT) is the most important biomarker described in GD. However, its clinical application is restricted because of a common genetic defect that results in the absence of detectable ChT in the plasma of 6% of the Caucasian population. Therefore, new surrogate biomarkers are needed. Proteomics has recently emerged as a new technology for global analysis of protein profiles and biomarker identification in biological fluids. This technology can play a critical role for monitoring response to therapies. In this work, we have studied 44 individuals for differentially expressed plasma proteins: 21 GD patients and 23 healthy controls. For patient sample collection, plasma was retrieved immediately following diagnosis and prior to treatment. Plasma samples were analysed using proteomic tools: proteins were resolved by two-dimensional gel electrophoresis (2-DE) and silver-stained. Image analysis was performed using Progenesis PG220 software. This analysis includes detection, quantification and normalization of each protein spot in every 2-DE image. Significant differences in protein expression levels between controls and GD patients were determined by t-Student test with a significance value of $p < 0.05$. Following this methodology, a set of 27 differentially-expressed plasma proteins has been identified by mass spectrometry. This set includes proteins involved in regulation of the immune system and inflammation (8 proteins), metabolism of lipoproteins (6 proteins), metabolism of iron (2 proteins), bone metabolism (1 protein), apoptosis (1 protein) and hormone transportation (1 protein). 2-DE results were validated by immunochemical methods (western-blot, ELISA and kinetic immunoturbidimetry). In conclusion, the proteomic technology applied to plasma samples is useful to identify plausible biomarkers for GD.

LENTIVIRAL VECTORS FOR GENE THERAPY OF GAUCHER DISEASE

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Introduction

Enzyme replacement and substrate reduction therapy for Gaucher disease are moderately effective in the majority of patients but have significant limitations in treating bone disease and neurological pathology. Gene therapy is an alternative approach, delivering a working copy of the affected glucocerebrosidase gene (GBA) to reconstitute the defective enzyme in patient's cells. We have experience of delivering genes to bone marrow haematopoietic stem cells (HSC) for gene therapy of inherited disease¹, and are applying this technology to Gaucher disease.

Cartier *et al.*² have halted the progression of adrenoleukodystrophy by injecting corrected HSCs into patients. These migrated to the brain and differentiated to microglia to express the missing protein. A similar approach may be effective for Gaucher disease.

Aims

Development of a viral vector to deliver a functional GBA gene to HSCs with the overall goal of correcting the metabolic defect in Gaucher cells.

Methods

The GBA cDNA was inserted into an HIV-1 based self-inactivating lentiviral vector and high titre virus was produced for testing. The virus was applied to the 293T immortalised cell line, type 1 patient derived leucocytes and type 2 patient-derived LCL cells (Coriell Institute for Medical Research). Enzyme production was confirmed by Western blot and measurement of enzyme activity using a clinical diagnostic assay.

Results

High titre virus was produced containing the GBA cDNA. 293T cells were efficiently transduced with this virus resulting in efficient over-expression of the gene and production of the protein. When applied to patient-specific primary cells, GBA enzyme activity was corrected to levels within the normal range. We also observed evidence of cross-correction of type 2 LCLs.

Discussion

We have produced a lentiviral vector capable of delivering a working copy of GBA which corrects enzyme activity levels in primary cells. We now aim to test this vector and therapeutic approach more thoroughly in cell lines and animal models.

¹ Gaspar, H.B. *et al.* (2004) *Lancet* **364**:2181-7

² Cartier, N *et al.* (2009) *Science* **326**:818-23

EFFICACY AND TOLERABILITY OF VELAGLUCERASE ALFA IN TREATMENT OF 7 PATIENTS WITH TYPE I GAUCHER DISEASE-FIRST OBSERVATIONS

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Introduction

Gaucher disease is the first lysosomal storage disease for which enzyme replacement therapy (ERT) has been successfully established. As a result of shortage of imiglucerase since June 2009, most Gaucher patients in Germany had a reduction or interruption of enzyme replacement therapy accompanied by the reemergence of clinical symptoms and disease progression. ERT with velaglucerase alfa in Germany started in the course of a "named-patient- program" in November 2009. In February of 2009, the FDA approved velaglucerase alfa enzyme replacement therapy (VPRIV®) for the indication of Type 1 GD in the US.

Patients and methods

From November 2009 to December 2009, 7 patients with reduced or interrupted ERT started velaglucerase alfa replacement therapy in doses between 30 to 60 U/kg body weight every 2 weeks. Prior to starting ERT with velaglucerase alfa, 5 of these patients had recent bone pain and/or a significant increase in the biomarker chitotriosidase; all of them showed changes of blood count. The clinical monitoring in the first weeks of treatment contained measurement of biomarkers and blood count as well as the disease severity scoring system GDS3 (Weinreb et al., Genet Med. Jan;12(1):44-51) and pain measurement by Visual Analog Scale (VAS).

Results

Following ERT with velaglucerase alfa 5 patients demonstrated a significant increase in platelet count and in 2 patients haemoglobin normalized within 4 months. One patient has a declined megaloblastic anemia by parenteral Vitamin B 12 supplement. Chitotriosidase declined in all patients, 2 patients had lower values than under treatment with imiglucerase. The measurement of pain by using the Visual Analog Scale (VAS) showed a significant reduction in pain in 2 patients, one patient became pain free. One patient reported fatigue on the day of ERT. Another experienced a modified thermal sensation for a few hours after therapy.

Conclusions

These results reflect first impressions and experiences in treatment with velaglucerase alfa in Type 1 GD. We have found that enzyme replacement therapy (ERT) with velaglucerase alfa is generally well tolerated with mild or no side-effects. Velaglucerase alfa was successful in improving reemergent clinical signs and symptoms in patients who had either come off or were on reduced doses of imiglucerase. In the opinion of the authors, ERT with velaglucerase alfa may, in some patients, be more efficacious than imiglucerase.

THE ASSESSMENT OF CARDIAC DISEASE WITH CARDIAC MAGNETIC RESONANCE IN GAUCHER PATIENTS

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Introduction

Gaucher disease (GD), the most common inherited lysosomal storage disorder, is a multisystemic disease due to an autosomal recessive defect of the gene encoding glucocerebrosidase enzyme, responsible for accumulation of glucosylceramide into reticuloendothelial cells, particularly in liver, spleen and bone marrow. The manifestations and the clinical progression of GD are highly variable between affected patients as well as age, features of clinical presentation and organ involvement. Each patient needs an accurate initial multisystemic assessment, staging the burden of the disease, followed by periodical evaluation. Cardiac disease is reported as a rare complication including restrictive cardiomyopathy and progressive calcifications of heart valves and aortic arch.

Aim

To assess the evidence of cardiovascular disease with CMR in GD patients.

Methods

Six consecutive patients were recruited at the Hereditary Anemia Center at Cà Granda Ospedale Maggiore Policlinico Foundation in Milan. In all patients, the diagnosis of GD was previously confirmed by enzymatic assay as well as by mutation analysis. Medical history of cardiac disease and the presence of cardiovascular risk factors were surveyed by direct interview. Patients were scanned with a 1.5 Avanto Siemens using a comprehensive cardiovascular evaluation protocol including morphologic T1 and T2-weighted sequences as well as functional cine sequences and inversion-recovery turbo-flash sequences to evaluate early and late-enhancement after gadolinium contrast media (Magnevist 0.03 mmol/Kg). Phase-contrast sequences were used to assess transvalvular velocities.

Results

Six patients were investigated, age 43 yrs (range 29-60 yrs), male, all receiving enzyme replacement therapy for a median of 8.5 years (range 1-18 yrs). Five patients showed bi-atrial enlargement (left atrium 4,5 cm, area 26 cm²), one patient showed moderate aortic stenosis (VENC=3.3 m/s) in bicuspid valve with mild aortic dilatation and one patient showed moderate mitral regurgitation (2/4). No evidence of myocardial enhancement was evident after gadolinium contrast media.

Discussion and conclusion

Although cardiac disease in GD is considered rare, in the present series we have found two valvular disease and mild to moderate bi-atrial enlargement in 5 of 6 patients, with a moderate increase of LV myocardial mass index. Further studies to evaluate the prognostic value of these findings are warranted.

EVALUATION OF A NEW GAUCHER DISEASE SEVERITY SCORING SYSTEM (DS3)

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Introduction

The aim of the study was to explore the application of a new disease severity scoring system in the cohort of Gaucher-patients from Children's Hospital of the Johannes-Gutenberg University Mainz. Preliminary results are presented.

Methods

56 patients from our clinic with Gaucher disease were analyzed in a retrospective study including clinical findings, medical history and personal interviews. Participating patients in the age-range of 8-60 years were followed with Aglucerase / Imiglucerase for 1 up to 15 years. The cohort was stratified into three subgroups with reference to the age at the diagnosis and start of therapy. The validated DS3 first introduced by Weinreb et al. was assessed at initiation of therapy (t_0) and as well at 1, 2, 5, 10 and 15 years of treatment. The score contains the domains bone disease, hematological and visceral. The score ranged from minimal 0 up to maximal 19 points, however > 9 points was considered as severe disease. For the patients under 18 years the category growth substituted the category of bone marrow infiltration. Furthermore the state of splenectomy, level of chitotriosidase and the dose of ERT were assessed.

Results

29 male and 27 female Gaucher patients were included in the analyses. In the group 1 (diagnosis and therapy as a child) were 23 persons, in the group 2 (diagnosis as a child, therapy as an adult) were 12 and in group 3 (diagnosis and therapy as an adult) 21 patients. At t_0 the score showed a median of 4,0 (25%: 3,0; 75%: 6,1). The median of the three groups at t_0 were 1: 3,4; 2: 5,2 and 3: 4,7. The score differs at t_0 and the other points in time (Wilcoxon-test, $p < 0,001$). The groups differs in the severity of the score in all points in time ($p < 0,003$ and $0,001$). The difference of score-points at t_0 and t_1 isn't statistically different between the groups, but of t_1 and t_2 (respectively t_2 and t_5 etc) ($p < 0,001$). The relative change of both the score-points and the level of chitotriosidase from t_0 to t_1 showed a mild correlation ($r = 0,339$, $p = 0,05$). There was no correlation between initial score-points and ERT-dose.

Discussion

As expected by clinical impression the improvement of disease-severity measured with DS3 in time is shown in the score. Also the difference of the three groups follows clinical experience as early treated children have less time to elaborate complications, however in this group the score is probably underestimated as growth retardation may not reflect completely bone involvement.

IMPACT OF TREATMENT WITH IMIGLUCERASE ON GLYCOSYLATED FERRITIN SERUM LEVEL IN GAUCHER DISEASE

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Introduction

Gaucher disease (GD) is a lysosomal storage disorder, caused by deficient activity of the enzyme glucocerebrosidase which can be treated by an enzyme replacement therapy (ERT). There is no prognostic marker that can predict long-term complications of GD but several markers are used in therapeutic monitoring: chitotriosidase, total serum ferritin (TSF), angiotensin-converting enzyme and tartrate resistant acid phosphatase. They all increase with the progression of the disease and generally decrease during treatment.

Aim

To investigate ferritin glycoforms, i.e. glycosylated ferritin (GF) and concentrations of non-glycosylated ferritin (NGF) as potential markers for the follow up of GD therapy.

Methods

GF and NGF levels evaluations of GD' patients followed in a single centre between 1996 and 2007, were analyzed. Two approaches were used: first, we compared the serum levels of 12 patients who did not receive therapy with that of 10 patients after 48 months on ERT; second, we analyzed the evolution of serum levels during ERT in 15 patients using linear mixed models.

Results

TSF and NGF levels were not significantly different between patients without treatment and patients on ERT (TSF: 524.5 µg/L [range 221.0–2045.0] versus 410.5 µg/L [range 115.0–1587.0] respectively, $P = 0.72$; NGF: 340.0 µg/L [range 182.8–1717.8] versus 199.9 µg/L [range 77.1–649.8], $P = 0.09$). The percentage of GF was significantly lower in patients without treatment than in patients on ERT (27.0 % [range 8.0–51.0] versus 43.5 % [range 22.0–80.0] respectively; $P = 0.02$).

GF percentage significantly increased during treatment (slope = 0.156 % per month, $P = 0.01$) whether NGF and TSF significantly decreased during treatment (slope = -3.13 µg/L per month, $P = 0.0002$; slope = -3.38 µg/L per month, $P = 0.002$, respectively).

Discussion

We showed that in GD the percentage of GF was low without treatment and significantly increased under ERT ; significant decrease in TSF and increase in percentage of GF during the course of ERT resulted in a significant highly decrease in absolute concentrations of NGF.

This is the first study that reported low percentage of GF in GD and showed an increase in GF during ERT. GF and NGF might be of clinical value for GD managing under treatment.

SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANTATION IN A PATIENT WITH GAUCHER DISEASE AND REFRACTORY HODGKIN'S LYMPHOMA.

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Introduction – Aims

Hodgkin's lymphoma (HL) is rare among patients with Gaucher disease (GD) and few cases have been reported. Moreover, only 2 cases of high-dose therapy (HDT) and autologous stem-cell transplantation (ASCT) have been reported. We describe the first case of a successful ASCT in a female patient with GD and repeatedly relapsed HL.

Case presentation

Type I GD had been diagnosed at the age of 15, she was splenectomized due to massive splenomegaly and started ERT 10 years later. At the age of 38, she presented with a painless left cervical mass, fever and sweats. A lymph node biopsy was interpreted as reactive lymphadenopathy but a second biopsy confirmed the diagnosis of nodular-sclerosing classical HL, stage IIIB. Treatment with 6 cycles of ABVD left residual disease, but subsequent radiotherapy resulted in complete remission (CR). Cervical lymphadenopathy reappeared 18 months later, but treatment with 4 cycles of DHAP was unsuccessful. A new lymph node biopsy confirmed the presence of refractory HL and 3 cycles of the DICE regimen resulted in a second CR, with a negative PET scan. An effort to mobilize and collect PBSC with the 4th cycle of DICE yielded 0.5×10^6 CD34+ cells/kg. A second effort for mobilization 2 months later was again unsuccessful, and meanwhile, a new relapse at the supraclavicular area appeared. Chemotherapy with 3 cycles of GVD (Gemcitabine, Vinorelbin and liposomal Doxorubicin) resulted in a third CR. A third mobilization course, by adding this time 2 doses of the anti-CXCR4 chemokine plerixafor, yielded a total of 3.6×10^6 CD34+ cells/Kg, in 2 days of collection. The patient was then offered HDT with BEAM and ASCT. She engrafted well, the posttransplant period was uneventful and 15 months later she remains in CR (PET-CT negative) and continues regular ERT.

Discussion

Diagnosis of HL may be difficult in patients with GD due to lymph node infiltration by Gaucher cells. Moreover, heavy bone marrow infiltration, splenomegaly and the chronic inflammatory reaction, might lead to severe chemotherapy-induced myelosuppression, delay the recommended dose administration and the scheduled time recycling, and contribute to treatment failure. Finally, difficulties in obtaining sufficient stem cell number and secondary graft failure have been reported in GD patients undergoing SCT. The possible contribution of GD per se, inducing an inflammatory marrow microenvironment, and impairing the growth of multipotent stem cells cannot be ruled out. This is the first described case of successful ASCT in a patient with GD and refractory HL, as well as the first case of successful mobilization with the use of plerixafor in a patient, in whom standard process was twice unsuccessful, suggesting that this drug may be effective in similar patients with lymphoproliferative disorders who are poor mobilizers.

MARKERS OF BONE METABOLISM:

Should the current concept on bone disease in Gaucher patients be remodelled?

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Introduction

Bone complications can be a prominent feature of Gaucher disease (GD) presenting as atypical bone pain, bone crises, avascular necrosis or pathological fractures. Because Gaucher cells release pro-inflammatory cytokines and acid phosphatase 5B, it has been hypothesized that bone disease results from increased macrophage mediated bone resorption. Enzyme replacement- and substrate reduction therapy (ERT, SRT) improve skeletal disease to some extent, but existing lesions persist. Thus, more insight in the pathophysiology is needed to effectively target bone complications.

Aim

To measure bone metabolism markers in a cohort of untreated GD patients in relation to clinical- and imaging data and review existing literature.

Methods

In 40 type I GD patients, of whom blood samples before or within one month of initiation of treatment were available, one bone resorption marker (type 1 collagen C-terminal telopeptide (CTX)) and two bone formation markers (N-terminal propeptide of type 1 procollagen (P1NP) and osteocalcin (OC)) were investigated. Correlation with imaging data including bone marrow fat fraction (QCSI), bone marrow burden (BMB) scores, bone mineral density measurements (DEXA), clinical data and chitotriosidase activity was studied. A Pubmed search using the words 'Gaucher disease' and 'bone metabolism' was conducted. Only articles on treatment-naïve GD patients or during ERT were selected.

Results

In the Dutch cohort CTX and P1NP were within the normal range for most patients (CTX median 225 ng/L, N<1008 ng/L) (P1NP median 37 ug/L, N= 19-96), while OC was decreased in 50% of our patients (median 0,35 nmol/L, N=0,4-4,0 nmol/L). OC concentration showed a positive correlation with QCSI scores (Rho 0,423; p 0,025) and both other bone markers (Rho 0,483; p 0,002 and Rho 0,466; p 0,002 for P1NP and CTX) and a negative correlation with chitotriosidase activity (Rho -0,323; p 0,042). P1NP and CTX showed no significant correlation with any of the imaging parameters. Of 11 studies in which bone metabolism markers were investigated, 6 were excluded because they focused on the effect of bisphosphonates or included a heterogeneous population. Bone formation markers were decreased in two studies and normal in another two. Bone resorption markers were inconsistent, with lower and higher levels compared to controls. During ERT, two out of three reports conclude that ERT increases bone formation parameters. None describes a significant effect on bone resorption markers.

Discussion

Our results suggest an imbalance in bone remodelling, characterized by a decrease in bone formation. We hypothesize that Gaucher cells (directly or indirectly) influence osteoblasts resulting in reduced bone formation and low osteocalcin levels. A reduction in Gaucher cell load could partly restore the imbalance and improve bone quality. Pharmacological agents that target bone formation might have additional effect.

**A PRELIMINARY ANALYSIS FROM THE ICGG GAUCHER REGISTRY:
Short-term Clinical Outcomes in Gaucher Patients Affected by the Shortage
of Imiglucerase**

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Introduction

Imiglucerase (Cerezyme[®]) is currently the only enzyme replacement therapy commercially available in Europe to treat patients with Gaucher disease. Beginning in June, 2009, a viral contamination at Genzyme's Allston Landing facility led to a significant reduction in the supply of imiglucerase, resulting in reduced dosing or complete interruptions in treatment for many patients. This regrettable shortage, however, does provide a unique chance to look into the impact of dose and dose frequency in the treatment of Gaucher disease. The ICGG Gaucher Registry collects information on clinical outcomes regardless of treatment status or choice.

Aim

Present a preliminary analysis plan for ICGG Gaucher Registry data to better understand the clinical impact of the imiglucerase supply shortage.

Methods

The Registry was queried to identify patients with Gaucher disease type 1 or type 3 with imiglucerase treatment data submitted both before and after the start of the supply shortage. June 25, 2009 was chosen as the shortage start date. Each patient's average dose (normalized to U/kg/2 weeks) before and after June 25, 2009 will be calculated. Overall time on treatment before and after June 25, 2009 will be calculated. Patients with treatment data will be categorized into quartiles according to the percentage reduction in dose, where the top quartile represents no or minimal change and the bottom quartile represents nearly complete interruption of treatment. Basic demographics and disease characteristics will be summarized. Patients with clinical data (hemoglobin, platelets, chitotriosidase, and bone pain/bone crisis) submitted to the Registry will be analyzed according to the change in each parameter from before to after June 25, 2009. Among patients with multiple data submissions per parameter, the latest data point during each time period (before/after June 25, 2009) will be used.

Results

An initial survey of data as of May 2010 identified 1282 patients with sufficient data for analysis. Analyses are ongoing.

Discussion

The data available in the ICGG Gaucher Registry are incomplete; due to the time delay associated with data entry, a full understanding of the impact on patients of the supply shortage is not yet possible. However we anticipate that awareness of this initial analysis plan may be useful to clinicians and may help Registry participants appreciate the value of continuing to enter data into the Registry even at this busy and challenging time.

P45

HIGH DENSITY LIPOPROTEIN CHOLESTEROL High (HDL-c) LEVELS IN TYPE I GAUCHER DISEASE

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Introduction

Low total cholesterol, low density lipoprotein (LDL) and high density lipoprotein(HDL-c) were found in Type I Gaucher disease (GD). These reported low levels of plasma lipoproteins correlated with the severity of GD clinical manifestations. It has been shown that enzyme replacement therapy (ERT) with Alglucerase, (Ceredase®) increased the levels of HDL-c, suggesting the reduction of the atherogenic effect.

Aim

Investigate the effect of ERT (Imiglucerase, Cerezyme®) on HDL-c levels and estimate the frequency of cardiovascular disease.

Methods

18 GD naïve patients and 21 on ERT were studied (approved by local IRB). HDL-c levels and cardiovascular events were monitored before and during ERT.

Results

HDL-c levels in untreated GD patients were 36.44 ± 9.38 mg/dL (median 35 mg/dL), while in patients on ERT HDL-c levels were significantly lower 26.23 ± 8.33 mg/dL (median 24 mg/dL) $p=0.001$. In GD patients on ERT (over 24 months) HDL-c levels increased from 25.33 ± 8.27 mg/dL (median 23.5 mg/dL) to 36 ± 10.98 mg/dL (median 38 mg/dL) $p < 0.001$. Only in 2/39 (5%) GD patients with low HDL-c suffered from cardiovascular events.

Conclusion

ERT results in an increase of HDL-c levels in GD patients. Despite low HDL-c a low frequency of cardiovascular events was found in this GD patients cohort.

ORAL PRESENTATION

Oral presentation number	Name	Thema	Institution
O1	Balreira, Andrea	Lysosomal integral membrane protein type-2 sorting receptor of β -glucocerebrosidase: a cell-type specific mechanism	Unidade de Biologia do Lisossoma e do Peroxissoma (UNLIPE), Universidade do Porto Portugal
O2	Svobodova, Eva	Glucocerebrosidase alternative promoter has features and expression characteristic of housekeeping genes	Institute of Inherited Metabolic Disorders, Charles University, 1st Faculty of Medicine and University Hospital Prague, Czech Republic
O3	Rahim, AA	Efficient CNS, PNS and Visceral Gene Delivery Following Fetal and Neonatal Intravenous Administration of AAV9 – An Approach for Studying Type II Neuronopathic Gaucher Disease	Institute for Women's Health, University College London, London, UK
O4	Alfonso, P.	Influence of c.(-203)>G variant in the glucocerebrosidase gene in type 1 Gaucher Disease phenotype	Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain
O5	Davies, E. H.	Diffusion Tensor Imaging: Study of brain white mater in paediatric Gaucher Type I and Type III	Institute of Child Health, University College, London and Great Ormond Street Hospital for Children NHS Trust, London, UK

O6	Lebel, E.	Osteonecrosis of the head of femur in Gaucher disease: a descriptive study of histological changes	Departments of Orthopedic Surgery and Pathology and the Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
O7	Pastores, Greg (Taskforce)	Helping to optimise patient care in Gaucher Disease: A novel assessment and monitoring tool for Therapeutic Goals	on behalf of the Therapeutic Goals Taskforce group
O8	Heidrich, Adina	Longterm Outcome with ERT in 26 German pediatric Gaucher patients	Villa metabolica, ZKJM MC Gutenberg University Mainz, Germany
O9	Ciana, G.	Bone mineral density responses to enzyme replacement therapy in pediatric patients	Regional Coordinator Centre for Rare Diseases, Institute of Hygiene and Epidemiology – University Hospital of Udine; Paediatric Clinic, I.R.C.C.S. “Burlo Garofolo”, Trieste; Italy
O10	Elstein, Deborah	Significant and continuous improvement in bone mineral density among type 1 Gaucher disease patients treated with velaglucerase alfa: 69-month experience, including dose reduction	Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
O11	Zimran, Ari	Experience in Australia & Israel with imiglucerase shortage by ‘drug holidays’ of ≤ 6 months in stable patients with type 1 Gaucher disease	Gaucher Clinic and Genetics Unit, Shaare Zedek Medical Center, Jerusalem, Israel

O12	Giraldo, P.	Spanish experience with Imiglucerase shortage	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain
O13	Saccari, Alessia	Clinical and biochemical surveillance program in a group of GD patients during shortage of ERT dosage	Regional Coordinator Centre for Rare Diseases, Institute of Epidemiology, University Hospital of Udine, Italy
O14	Stirnemann, Jerome	Imiglucerase Shortage: The French Experience	Dept of Internal Medicine, Hôpital Jean-Verdier, Assistance Publique-Hôpitaux de Paris, Université Paris XIII, France
O15	Hughes, Derralynn	United Kingdom Experience of 38 adult Gaucher patients switched to enzyme replacement therapy with Velaglucerase as a result of the international Imiglucerase shortage	Lysosomal Storage Disorders Unit, Royal Free Hospital, London, and Lysosomal Disorders Unit, Addenbrooke's Hospital, Cambridge, UK
O17	Böttcher, Tobias	Early signs of Parkinson's disease in Gaucher patients	Albrecht-Kossel-Institute for Neuroregeneration, University of Rostock, Germany
O18	Davies, E. H.	Four year follow up of Type III Gaucher patients using a modified Severity Scoring Tool	Institute of Child Health, University College London; Children's Hospital, University of Mainz, Germany; Child Health Institute, Poland; Vilnius University Childrens Hospital, Vilnius, Lithuania and Great Ormond St Children's Hospital, London, UK

O19	Biegstraaten, M.	Type 1 Gaucher disease patients exhibit cognitive function deficits: results of a two-year prospective observational study	SPHINX Amsterdam Lysosome Centre, Academic Medical Centre, Amsterdam, The Netherlands
O20	Mengel, Eugen	Neuronopathic Gaucher disease: Follow Up and Longterm Outcome in 30 German Patients	Villa metabolica, ZKJM MC Gutenberg University Mainz, Germany
O21	Ron, I.	Parkin-mediated ubiquitination and degradation of mutant glucocerebrosidase variants-a possible link between GD and Parkinson disease	Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, 69978, Israel
O22	Goker-Alpan, O.	The Link between innate immunity and Gaucher disease	LSD Research and Treatment Unit, Center for Clinical Trials, O&O Alpan LLC, Springfield, VA, USA
O23	van Eijk, Marco	Reducing glycosphingolipids restores insulin sensitivity in obese mice	Dept of Medical Biochemistry and Dept. Of Pathology, AMC, University of Amsterdam, The Netherlands
O24	Yildiz, Y.	Role of GBA2 in Morbus Gaucher	Department of Internal Medicine I, University Clinic of Bonn, Germany

O25	Mistry, Pramrod	GBA1 Deficient Mice Recapitulates Gaucher's Disease Displaying System-wide Cellular and Molecular Dysregulation Beyond the Macrophage: Evidence for osteoblastic bone formation defect underlying osteopenia	Departments of Pediatrics, Medicine, Comparative Medicine, Genetics and Pathology, Yale School of Public Health, Keck Center for Genomics, Yale School of Medicine, New Haven, CT 06562, USA
O26	Chung, Y.	Imiglucerase biosimilar development for Gaucher disease	ISU Abxis Co., Yonsei University Medical School ,134, Shinchon-dong, Seodaemun-gu, Seoul, 120-752, South Korea
O27	Zimran, Ari	Novel Enzyme Replacement Therapy for Gaucher Disease: Phase III Pivotal Clinical Trial with Plant Cell Expressed Recombinant Glucocerebrosidase (prGCD) - taliglucerase alfa	Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
O28	Gonzalez, D.	Enzyme replacement therapy with velaglucerase alfa significantly improves key clinical parameters in type I disease: positive results from a randomized double-blind, global phase III study	Sanatorio Español, Asunción, Paraguay
O29	Poll, L.	Whole body MRI in type I Gaucher patients: preliminary results of bone involvement	Department of Radiology, Berufsgenossenschaftliche Unfallklinik Duisburg GmbH, Duisburg, Germany
O30	Ruiz, Juan	Antigenic differences in patients with type 1 Gaucher disease receiving velaglucerase alfa or imiglucerase enzyme replacement therapy in controlled clinical trials	Shire Human Genetic Therapies, Cambridge, MA, USA

O31	Giraldo, P.	Safety and efficacy of velaglucerase alfa in Gaucher disease type 1 patients previously treated with imiglucerase	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza and Hospital Universitario Miguel Servet, Zaragoza, Spain
O32	Kallemeijn, W. W.	A novel, ultra-sensitive technique to visualize active glucocerebrosidase	Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
O33	Lukina, E.	Eliglustat Tartrate, An Investigational oral COMPOUND for Gaucher Disease Type 1 (GD1): Phase 2 Results after 2 YearS	National Research Center for Haematology, Moscow, Russia and Genzyme Corporation, Cambridge, MA, USA
O34	Luan, Z.	SP2-lminisugars as pharmacological chaperones for Gaucher disease: mutation profiling, cellular uptake and intracellular distribution studies	Div. Of Child Neurology, Inst. Of Neurological Sciences, Fac. Of Medicine, Tottori University, Nishi-cho, Yonago, 683-8504 Japan

ABSTRACTS

Abstract number	First author	Title	Institution
1	Alfonso, P.	Therapeutic Strategies for Gaucher Disease: Different thermostability of Velaglucerase alfa and Imiglucerase	Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain
2	Altarescu, G.	Preimplantation Genetic Diagnosis (PGD) for a treatable disorder: Gaucher disease type I as a model	Medical Genetics Institute, Zohar PGD Lab; IVF Unit, and Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
3	Ayto, RM	Gaucher derived monolayers prime plasma cells for survival against chemotherapeutic agents	Royal Medical School, Haematology
4	Benediktov-Bar, I.	ER-associated degradation of the L444P mutant glucocerebrosidase variant	Department of Cell Research and Immunology, Tel Aviv University, Ramat Aviv, 69978, Israel
5	Bettman, Noam	Dendritic cell dysfunction in patients with Gaucher disease	Bruce Rappaport Faculty of Medicine, Technion, Institute of Technology, Haifa, Israel
6	Biegstraaten, M.	Peripheral neuropathy in adult type 1 Gaucher disease: A two-year prospective observational study	Academic Medical Centre, Amsterdam, The Netherlands

7	Bokareva, M.	Iron metabolism in treatment-naïve Gaucher patients in Russia	National Research Centre for Hematology, Moscow, Russia
8	Bracoud, Luc	Measurement of spleen and liver volumes using MRI in an enzyme-replacement therapy multi-center trial on naïve patients with Gaucher Disease	BioClinica, Protalix Biotherapeutics
9	Bratosin, Daniela	A cytometric study of the red blood cells in Gaucher disease suggests that they may be involved in increased erythrophagocytosis	National Institute for Biological Science Research and Development, Bucharest and "Vasile Goldis" Western University of Arad, Faculty of Natural Sciences, Arad, Romania
10	Cappellini, M.D.	Determinants of persisting thrombocytopenia in patients with type 1 Gaucher disease treated with alglucerase/ imiglucerase for 4-5 years	Department of Internal Medicine, Policlinico Foundation IRCCS, University of Milan, Italy
11	Church, H.J.	Mutations frequencies in Gaucher's disease in different populations	Willink Unit, Genetic Medicine, St Mary's Hospital, Manchester M13 9WL, UK
12	Davies, E. H.	A modified Severity Scoring Tool for Neuronopathic Gaucher disease	1Institute of Child Health, University College London. 2Great Ormond Street Hospital for Children NHS Trust, London, UK
13	Davies, E. H.	Quantifying ataxia in Neuronopathic GD	Institute of Child Health, University College London, Great Ormond St Children's Hospital, London, UK

14	Dimitriou E.	The frequency of the N370S mutation in the Greek population	1Institute of Child Health, "Aghia Sophia" Children's Hospital, Athens, Greece, Dept Biological Chemistry, Medical School, Athens University, Athens, Greece
15	Donner, Markus	Management of a patient with non-neuropathic Gaucher disease diagnosed in pregnancy	Department of Gastroenterology, Hepatology and Infectious Diseases, University of Düsseldorf, Germany
16	Elstein, Deborah	Achievement of long-term therapeutic goals for enzyme replacement therapy (ERT) in patients with Gaucher disease type 1 receiving velaglucerase alfa	Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
17	Garcia-Rodriguez, B.	Incidence of GBA mutations in Parkinson's disease patients in Aragon (Spain)	Translational Research Unit, Miguel Servet University Hospital, Zaragoza, Spain
18	Gervas-Arruga, J.	Proinflammatory bone cytokine profile in Gaucher disease	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain
19	Giraldo, P.	Clinical, genetic characteristics and geographical distribution of Gaucher Disease in Iberian Peninsulae	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza & Barcelona, Spain
20	Giraldo, P.	Safety with velaglucerase in two girls previously treated with imiglucerase	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain

21	Granovsky-Grisaru, S.	Updated recommendations for the management of pregnancy in Gaucher disease	Dept of Obstetrics and Gynecology and Gaucher Clinic, Shaare Zedek Medical Center, Hebrew University, Jerusalem, Israel
22	Irún, P.	Genotyping pitfalls in a family with Gaucher disease	Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Zaragoza, Spain
23	Jones, C.	RegistryNXT!: New technology to enhance data collection and reporting for the Lysosomal Storage Disorders Registries	Genzyme Corporation, Cambridge MA, USA and Genzyme Europe, Naarden, The Netherlands
24	Jurecka, Agnieszka	Dysgammaglobulinemia in Gaucher disease: data from 61 patients, including 18 patients with GD type III	Department of Metabolic Diseases, Endocrinology and Diabetology, The Children's Memorial Health Institute, Warsaw, Poland
25	Kulikov, V.	Ph-positive chronic myeloid leukemia in a patient with Gaucher disease	National Research Center for Haematology, Moscow, Russia
26	Lebel, E.	Bone density changes in children with Gaucher disease: A proposal for an international collaborative effort	Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel
27	Lukina, Kira	Clinical data of splenectomized and non-splenectomized patients in Russia	National Research Center for Hematology, Moscow, Russia

28	Malini, E.	Identification and functional characterization of 9 novel mutant alleles causing Gaucher disease	Regional Coordinator Centre for Rare Diseases. University Hospital "Santa Maria della Misericordia", Udine, Italy
29	Milligan, A	Velaglucerase as Enzyme Replacement Therapy in type 1 Gaucher Disease: Reporting the first UK non-trial patient	Lysosomal Storage Disorders Unit, Royal Free Hospital, London. 2010, UK
30	Mitrovic, Mirjana	Platelet function and coagulation abnormalities in type 1 gaucher patients: effects of imiglucerase therapy	Institute of Hematology CCS, Belgrade, Serbia
31	Moraitou, M.	GBA mutations and Parkinson's disease	Institute of Child Health, Athens, Greece; Laboratory of Neurogenetics, Dept. Neurology, Medical School, University of Thessaly, Greece; Div. Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens and 2nd Dept of Neurology, University of Athens Medical School, Greece
32	Moss, G.	Wellbeing of Manchester patients who changed over from Imiglucerase (Cerezyme) to Velaglucerase in the hospital and the home settings in children with Type 1 and Type III Gaucher disease	Department of Genetic Medicine St Marys Hospital Central Manchester University Hospitals NHS Trust, Manchester, UK
33	Oz, A.	Home infusions provide a convenient option for intravenous enzyme replacement therapy with velaglucerase alfa in patients with type 1 Gaucher disease	Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel

34	Peterson, B.	Comparison of In Vitro Cellular Uptake of velaglucerase alfa to that of imiglucerase: Effects of Chemical Parameters and Receptor-Specific Inhibition	1Department of Research and Development, Shire Human Genetic Therapies, 700 Main Street, Cambridge, MA, 02139, USA
35	Poll, L.	MRI bone marrow findings in 63 patients with type I Gaucher disease	1Department of Radiology, Berufsgenossenschaftliche Unfallklinik Duisburg GmbH, Grossenbaumer Allee 250, D-47249 Duisburg, Germany
36	Quintana, L.	Identification of biomarkers of Gaucher disease using proteomic tools	Instituto Aragonés de Ciencias de la Salud, Zaragoza, Spain
37	Rahim, AA	Lentiviral vectors for gene therapy of Gaucher disease	UCL Institute for Women's Health, London, UK
38	Reinke, J.	Efficacy and tolerability of velaglucerase alfa in the treatment of 7 patients with type I Gaucher disease-first observations	Children's Hospital, Gutenberg-University of Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany
39	Roghi, A.	The assessment of cardiac disease with Cardiac Magnetic Resonance (CMR) in Gaucher patients	CMR Unit, Department of Cardiology, Niguarda Ca' Granda Hospital, Milan, Italy
40	Schöttler, Hanna	Evaluation of a new Gaucher disease severity scoring system (DS3)	Villa metabolica, ZKJM MC Gutenberg University Mainz, Germany

41	Stirnemann, Jerome	Impact of treatment with imiglucerase on glycosylated ferritin serum level in Gaucher disease.	Médecine Interne, Hôpital Jean-Verdier, Assistance Publique-Hôpitaux de Paris, Université Paris XIII, Referral Center of Lysosomal Diseases (RCLD), Bondy, France
42	Symeonidis, Argiris	Successful autologous stem cell transplantation in a patient with Gaucher disease and refractory Hodgkin´s lymphoma	Hematology Division, Dept of Internal Medicine, University of Patras Medical School Patras, Greece
43	van Dussen, Laura	Markers of bone metabolism: should the current concept on bone disease in Gaucher patients be remodelled?	Dept. of Endocrinology and Metabolism, Academic Medical Center Amsterdam, The Netherlands
44	vom Dahl, Stephan	A Preliminary Analysis from the ICGG Gaucher Registry	St. Franziskus-Hospital, Cologne, Academic Teaching Hospital, University of Cologne, Germany
45	Watad, Salmas	High density lipoprotein cholesterol (HDL-c) levels in Type I Gaucher disease	Department of Hematology & Bone Marrow Transplantation; Rambam Health Care Campus, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

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Stübinger	Department of Internal Medicine	St. Franziskus Hospital, Academic Teaching Hospital of the University of Cologne	Germany
Sumarac		Institute of Medical Biochemistry, CCS	Serbia

Sun	The Mount Sinai Bone Program	Mount Sinai School of Medicine	USA
Suvajdzic		Institute of Hematology CCS	Serbia
Suzuki		Int. University of Health and Welfare Graduate School	Japan
Svobodová	Institute of Inherited Metabolic Disorders	Charles University, 1st Faculty of Medicine and University Hospital	Czech Republic
Symeonidis	Hematology Division, Dept of Internal Medicine	University of Patras Medical School	Greece
Sysoeva		National Research Center for Hematology	Russia
Szer	Director, Department of Clinical Haematology & BMT Service	Royal Melbourne Hospital	Australia
Terreros Muñoz		Centro Médico Nacional Siglo XXI Departamento Clínico de Hematología	Mexiko
Tiniakou	Hematology Division, Dept of Internal Medicine	University of Patras Medical School	Greece
Tissier	Domaine du CERTIA	Station de l'INRA	France
Triantafyllou	Hematology Division, Dept of Internal Medicine	University of Patras Medical School	Greece
Troungos	Dept Biological Chemistry	Medical School, Athens University	Greece
Turkia		La Rabta Hospital	Tunesia
Turkina		National Research Center for Haematology	Russia
Tylki- Szymanska	Department of Metabolic Diseases, Endocrinology and Diabetology	The Children's Memorial Health Institute	Poland
Unutmaz		Laboratory of Immunology/T cell interactions, NYU	USA
Walter	Department of Neurology	University of Rostock	Germany
van der Marel	Department of Bio-organic Synthesis	Leiden Institute of Chemistry	The Netherlands
van Dussen	Dept. of Endocrinology and Metabolism	Academic Medical Center	The Netherlands
van Eijk	Dept of Medical Biochemistry	University of Amsterdam	The Netherlands
van Gool	SPHINX Amsterdam Lysosome Centre	Academic Medical Centre	The Netherlands
van Roomen	Dept of Medical Biochemistry	University of Amsterdam	The Netherlands
van Schaik	SPHINX Amsterdam Lysosome Centre	Academic Medical Centre	The Netherlands
Varshower	Zohar PGD Lab; IVF Unit	Shaare Zedek Medical Center	Israel
Velázquez-Campoy		Aragon Health Sciences Institute (I+CS)	Spain
Vellodi		Great Ormond Street Hospital for Children NHS Trust	UK

Villageliu		Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)	Spain
Vincent	Laboratoire de biostatistiques, INSERM	Hôpital Bichat, Université Paris-Diderot	France
Virkar		Digital Infuzion	USA
vom Dahl	Department of Internal Medicine	St. Franziskus Hospital, Academic Teaching Hospital, University of Cologne	Germany
Vrins	Dept of Medical Biochemistry	University of Amsterdam	The Netherlands
Waddington	Institute for Women's Health	University College London	UK
Wang		Shire HGT	USA
Watad	Department of Hematology & Bone Marrow Transplantation	Rambam Health Care Campus, Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology	Israel
Watman		Hospital Ramos Mejia	Argentina
Weinberg	Center of Oncology		Germany
Weinreb		University Research Foundation for Lysosomal Storage Disorders	USA
Wesnes		United BioSource Corporation	UK
Wickremasinghe			
Willers	Research Centre	Heinrich Heine University	Germany
Wilson	Department of Clinical Genetics	Children's Hospital at Westmead, and Disciplines of Paediatrics and Child Health & Genetic Medicine, University of Sydney	Australia
Witte	Department of Bio-organic Synthesis	Leiden Institute of Chemistry	The Netherlands
Wong	Paediatric Storage Disease Laboratory	Kings College London	UK
Wood		Great Ormond Street Hospital for Children NHS Trust	UK
Wraith	Willink Unit, Genetic Medicine	St Mary's Hospital	UK
Xiromeriou	Laboratory of Neurogenetics, Dept. Neurology	Medical School, University of Thessaly	Greece
Yee		Genzyme Corporation	USA
Yildiz	Department of Internal Medicine I	University Clinic of Bonn	Germany
Yim	ISU Abxis Co.	Yonsei University Medical School	South Korea
Zahrieh		Shire Human Genetic Therapies	USA
Zaidi	The Mount Sinai Bone Program	Mount Sinai School of Medicine	USA
Zhang		Genzyme Corporation	USA

Zimran	Gaucher Clinic	Shaare Zedek Medical Center Hebrew University	Israel
Zoumbos	Hematology Division, Dept of Internal Medicine	University of Patras Medical School	Greece

Further Informations

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Geschäftsstelle der ASIM

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Hotels

CLASSIC

Geno Hotel

Raiffeisenstraße 10
51503 Rösrath
Fon +49 (0)2205 803-0
www.genohotel.de
Distance to conference location: 6 kilometers

Kardinal Schulte Haus

Overather Straße 51
51429 Bergisch Gladbach
Fon +49 (0)2204 408-0
www.k-s-h.de
Distance to conference location: 1 kilometer

Hotel im Goethehaus

Markt 3
51429 Bergisch Gladbach-Bensberg
Fon +49 (0)2204 2059-0
www.hotel-goethe-haus.de
Distance to conference location: 0.5 kilometers

EXECUTIVE

Grandhotel Schloss Bensberg

Kadettenstraße 1
51429 Bergisch Gladbach
Fon +49 (0)2204 42-0
www.schlossbensberg.com
Distance to conference location: 0 kilometers

