PERIPHERAL NEURAL RESPONSE AND SEX HORMONES IN TYPE 1 GAUCHER DISEASE

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 Summary

Background: In a rare Gaucher disease, reduced activity of lysosomal β-glucocerebrosidase incompletely blocks glucosphingolipid catabolism. Accumulation of the unhidrolized substrate glucosylceramide within lysosomes results in progressive, multisystem Gaucher disease, classified into three types. Both parkinsonism and peripheral neuropathy are observed in cases of putative non-neuroopathic type 1 disease. In the current study we investigated whether the peripheral neural response in type 1 Gaucher disease patients, with no neural manifestations is conditioned by the influence of sex hormones.

Methods: The catalytic activity of β-glucocerebrosidase in peripheral blood leukocytes was determined spectrofluorometrically. Direct sequencing of the GBA1 gene was performed. Somatosensory evoked potentials were recorded after electrical stimulation of the median nerve of both arms. Stimuli of 0.2 ms duration at a frequency of 5 Hz were used. Sex hormones were determined by radioimmunoassay using a gamma scintillation counter.

Results: Analysis of the somatosensory evoked potentials revealed significant differences in peak latencies on periphery between men and women in both control and type 1 Gaucher disease groups. Analysis by gender showed significant associations between latencies and sex hormones.
Introduction

Gaucher disease (GD) is a rare lysosomal storage sphingolipidosis caused by compound heterozygous or homozygous mutations in the GBA1 gene (Online Mendelian Inheritance in Man (OMIM) #606463) (1). Consecutive unnatural configuration and insufficient activity of lysosomal β-glucocerebrosidase leads to incomplete block in glucosphingolipid catabolism (2, 3). Since sphingolipids and their metabolites, as structural elements of membranes and signaling molecules, play a vital role in cell physiology, accumulation of the unhydrolyzed substrate glucosylceramide within lysosomes results in progressive, multisystem GD with a wide phenotypic spectrum (4). GD is classified into mandatory non-neuropathic type 1 (OMIM #230800), acute neuropathic type 2 (OMIM #230900) and chronic neuronopathic type 3 (OMIM #231000) disease. However, the occurrence of parkinsonism and peripheral neuropathy different from the specific characteristics defined by type 2 and 3 has been observed in a number of patients with type 1 GD (5, 6).

The aim of this study was to determine whether the peripheral neural response in type 1 GD is under the influence of sex hormones.

Materials and Methods

Patients

In this cross-sectional study, 20 type 1 GD patients with no clinical neurological manifestations (10 women and 10 men) aged 19 to 61 years were compared with healthy controls matched for gender and age. Among the patients, three were treatment-naive, fourteen had received enzyme replacement therapy and three substrate-reducing therapy for more than 5 years.

Compliance with ethical standards

Approval for the study was obtained from the Ethics Committee of the Medical Faculty, University of Belgrade (decision number 29/III-12 from 27.03.2015.).

Conclusions: A relationship between testosterone concentrations and the latencies of potentials evoked on peripheral nerves exists only in females with type 1 Gaucher disease. We point out sexual dimorphism in the development of this entity.

Keywords: Type 1 Gaucher disease, somatosensory evoked potentials, latencies, oestradiol, testosterone

Data acquisition methods

The algorithm for setting the GD diagnosis included clinical findings, assessment of biomarker activity followed by determination of specific enzyme activity and was confirmed by identification of GBA1 gene mutations.

Biochemistry

The activity of the biomarker chitotriosidase in serum was measured spectrofluorometrically using the fluorogenic substrate 4-methyl-umbelliferyl-β-N-N’-N”-triacetetylchitotrioside (Sigma Chemical Co, USA) on an SPF-500™C spectrofluorometer (SLM Instruments Inc, USA). The fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside (Sigma Chemical Co, USA) was used for determining the catalytic activity of β-glucocerebrosidase in peripheral blood leukocytes spectrofluorometrically using the same spectrofluorometer.

The following ranges of control values (as 2.5 and 97.5 percentiles) for a healthy population were established at the Clinical Center of Serbia: for chitotriosidase activity 1.80–146.56 nmol/mL/h; for β-glucocerebrosidase activity 6.65–15.90 nmol/mg/h.

Molecular genetics

The GBA1 gene was analyzed by direct sequencing (Applied Biosystems 3500, Hitachi, USA).

Neurophysiology

Somatosensory evoked potentials (SSEP) were recorded after electrical stimulation of the right and left median nerve at the wrist (MedelecSynergy, Viasys Healthcare, UK). Stimuli of 0.2 ms duration were given at a frequency of 5 Hz.

Hormones

Oestradiol (E2) (ESTR-US- CT, Cisbio Bioassays, France, coefficient of variance CV, 2.8%) and testos-
testosterone (TESTO- CT2, Cisbio Bioassays, France, CV 3.1%) were measured by radioimmunoassay using a gamma scintillation counter (CliniGamma 1272, LKB-Wallac, USA).

Statistics

Normal distribution of the data was examined by the Shapiro-Wilk test. We used two way ANOVA (group, gender) to assess differences between the control and type 1 GD groups. Pearson’s correlation coefficient was employed to estimate linear relationships between the variables. Data are presented as means ± standard error. Differences were considered statistically significant at $p < 0.05$. We used SPSS 19 (IBM) software for statistical analysis.

Results

Biochemistry

Pretreatment chitotriosidase activity levels ranged from 2404 to 26930 nmol/mL/h. Residual levels of $\beta$-glucocerebrosidase activity were between 0.50 to 3.14 nmol/mg/h.

Molecular genetics

Two patients were homozygous for the GBA1 mutation c.1226A>G, while all the others had compound heterozygous mutations. In each control participant, the wild type GBA1 gene was confirmed in both alleles.

Anthropology

There was no age difference between the groups (Table I). In terms of height, significant differences existed in each group: women were shorter than men in both groups; women with type 1 GD were shorter than the control women ($p = 0.03$) (Table I).

| Table I Anthropological data and sex hormones levels. |
|------------------------------------------------------|
|                                                      |
| Control (N = 20)                                      |
|                                                      |
| Gaucher (N = 20)                                      |
|                                                      |
| Women | Men | $p$ | Women | Men | $p$ | $p$ (Con vs. GD) |
|--------|-----|----|--------|-----|----|-----------------|
| Age (years) | 36.5 ± 4.4 | 0.19 | 38.2 ± 4.1 | 0.52 | 0.93 |
| Height (cm)  | 171.5 ± 1.3 | 0.01 | 164 ± 2.7 | 0.01 | 0.24 |
| Testosterone (nmol/L)  | 1.07 ± 0.12 | 0.01 | 0.89 ± 0.17 | 0.01 | 0.63 |
| Oestradiol (pmol/L) | 252 ± 60 | 0.06 | 274 ± 76 | 0.07 | 0.88 |
|                                                      |
| Data are presented as mean values ± standard error.  |

Figure 1 Peak latencies plus standard error in control women (CW), control men (CM), type 1 GD female patients (GDW), type 1 GD male patients (GDM), * $p < 0.01$.

Figure 2 Relationship between peak latency N13 and plasma testosterone levels in GDW (solid circle), CW (open circle), GDM (solid square) and CM (open square).
SSEP

Regarding the average latency values there were no difference for all parameters between the left and right median nerves (latency of the N9, N11 and N13 waves), no when the control and GD groups were compared in total and for each gender separately. However, within each groups, a significant differences in peak latencies between men and women were identified from N9 to N13 (p ≤ 0.01) (Figure 1).

Sex hormones

As expected both groups showed statistically significant differences in testosterone concentrations between women and men (Table 1). There was a tendency for the difference between the sexes for oestra-
diol concentration to approach statistical significance in both groups (Table 1).

Correlations

Statistically significant latency correlations with the height of subjects were present only in women with type 1 GD: N9 (r = 0.78, p < 0.01), N11 (r = 0.58, p = 0.08, near), N13 (r = 0.70, p = 0.03).

Considering men and women together, there were significant positive correlations between all latencies and testosterone concentrations in both the control and type 1 GD groups: N9 (r = 0.57 vs. r = 0.58, p < 0.01, respectively), N11 (r = 0.60 vs. r = 0.62, p < 0.01, respectively), and N13 (r = 0.65 vs. r = 0.67, p < 0.01, respectively) (Figure 2).

However, when the analysis was performed for each gender, significant latency correlations with sex hormones were identified only in female patients with type 1 GD: negative correlation between estradiol concentration and N9 peak latency (r = -0.63, p = 0.05) and negative correlations of testosterone levels with all peak latencies on the periphery N9 (r = -0.58, p = 0.08, tendency), N11 (r = -0.76, p = 0.01), N13 (r = -0.75, p = 0.01).

Discussion

A crucial large-scale prospective observational cohort study, employing strictly defined criteria, provided evidence for polyneuropathy as part of the natural course of type 1 GD (6). In 103 patients enrolled at 18 to 75 years old, 13.6% were untreated and 86.4% received enzyme replacement therapy. Among them, 10.7% were diagnosed with sensory motor axonal polyneuropathy at baseline using standardized electro-
physiological assessment. Six new cases of polyneuropathy were revealed during two-years monitoring (2.9 per 100 person-years). The same diagnostic pro-
cedure was used for the 25 healthy subjects. Since prevalence and incidence of polyneuropathy in the general population were estimated to be between 0.09 and 1.3% and 0.0046 and 0.015 per 100 person-years, respectively, it was concluded that both prev-
ance and incidence of polyneuropathy in type 1 GD patients are greater than for the general population. Therefore, for this study we enrolled only patients with no peripheral neurological manifestations.

Until now, only one multimodal neurophysiological investigation (including SSEP) has been performed in adult subjects with type 1 GD (7). It involved eight female and four male adult patients aged 17 to 48 years. Findings were obtained from the right medi-
an nerve. In all subjects normal recordings from the periphery (N9, N11, N13) were read out. We have confirmed such normal findings but have gone further. Namely the present cross-sectional SSEP study on the median nerve periphery revealed gender differences – shorter latency peaks in women from both groups (healthy, diseased) which has not been noticed, so far.

Neuroactive steroids include those produced by the nervous system and hormones originating from the gonads and adrenal glands. The peripheral nervous system (PNS) not only synthesizes and metabolizes neuroactive steroids, but peripheral nerves also express receptors for neuroactive steroids and, therefore, are a target for their activity (8, 9). Steroids acting in the nervous system realize their effects via classical intra-
cellular androgen, progesterone, oestrogen, glucocorticoid and mineralocorticoid receptors, as well as via non-classical steroid receptors expressed by different cellular components of the PNS (10, 11). Therefore, regulating PNS physiology over various signaling path-
ways, neuroactive steroids, including testosterone, can influence different peripheral nerves functions, among which Schwann cells proliferation and myelination have been studied in particular (8, 11).

Striking sexual dimorphism of white matter growth in adolescent brains has already been observed, but not explained (12, 13). In order to assess the role of the androgen receptor (AR) in mediating the effect of testosterone on white matter growth, 204 male and 204 female adolescents were studied (14). Functional polymorphism in the AR gene (number of CAG repeats in exon 1) was geno-
typed, together with measurement of plasma testosterone concentration, computational analysis of magnetic resonance images and calculation of the magnetization transfer ratio (MTR) for white matter throughout the brain as an indirect index of myelination. Evidence emerged that a genetic variation in the AR gene moderates the effect of testosterone on white matter volume, 204 male and 204 female adolescents were studied (14). Functional polymorphism in the AR gene (number of CAG repeats in exon 1) was geno-
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explained by an increase in myelination. It was assumed that testosterone effected axonal diameter, rather than myelin sheath thickness. The direct consequence of increased axonal diameter under the influence of testosterone is a decrease in the number of fibers per unit volume, which is manifested as a lower index of myelination and lower MTR values (14). Likewise, the latter might explain the peripheral nerve response data obtained in the current study. Within the total testosterone concentration range (males plus females), the positive correlation between plasma testosterone levels and all peak latencies in all participants in each group is very significant. Since the number of nerve fibers is a key factor determining the efficiency of peripheral nerve transmission (15), the smaller number of myelinated fibers in the median nerve, due to their large testosterone-induced caliber in men, could be the reason for longer latencies.

When females were analyzed per se, using the lower range of testosterone values in women (up to 1.9 nmol/L), only female patients with type 1 GD showed a statistically significant negative correlations of plasma testosterone levels with latencies from N9 to N13. This association indicates that a normal SSEP finding alone is not sufficient to draw conclusions about undisturbed morphological and functional nerve integrity on the periphery in type 1 GD females. Why small changes in testosterone concentrations in women with type 1 GD affect the duration of impulse propagation should be clarified. Experimental models have indicated that pathology affects the concentration of neuroactive steroids present in peripheral nerves in a sex-dimorphic way (16, 17).

We have entered a serious area that has yet to be examined in detail due to insufficient available data on this matter.

**Conflict of interest statement**

The authors state that they have no conflicts of interest regarding the publication of this article.

**References**

1. Grabowski GA, Horowitz M. Gaucher's disease: molecular, genetic and enzymo-logical aspects. Baillieres Clin Haematol 1997; 10(4): 635–56.
2. Brady RO, Kanfer JN, Shapiro D. Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. Biochem Biophys Res Commun 1965; 18(2): 221–5.
3. Vitner EB, Platt FM, Futerman AH. Common and uncommon pathogenic cascades in lysosomal storage diseases. J Biol Chem 2010; 285(27): 20423–7.
4. Rodić P, Lakočević M, Pavlović S, Đurašević TK, Kostić T, Vukočević NS, Šumarac Z, Petakov M, Janić D. Immunoglobulin heavy chain gene rearrangements in patients with gaucher disease. J Med Biochem 2018; 37: 307–12.
5. Neudorfer O, Giladi N, Elstein D, Abrahavov A, Turez-kite T, Aghai E, Reches A, Bembali B, Zimran A. Occurrence of Parkinson's syndrome in type I Gaucher disease. QJM 1996; 89(9): 691–4.
6. Biegstraaten M, Mengel E, Marodi L, Petakov M, Niederau C, Giraldo P, Hughes D, Masic M, Mehta A, Hollak CE, van Schaik IN. Peripheral neuropathy in adult type 1 Gaucher disease: a 2-year prospective observational study. Brain 2010; 133(10): 2909–19.
7. Perretti A, Parenti G, Balbi P, Titomanlio L, Marcantonio L, Iapoce M, Frascogna AR, Andria G, Santoro L. Study of multimodal evoked potentials in patients with type 1 Gaucher's disease. J Child Neurol 2005; 20(2): 124–8.
8. Melcangi RC, Cavarretta IT, Ballabio M, Leonelli E, Schenone A, Azzocchia I, Miguel Garcia-Segura L, Magnaghi V. Peripheral nerves: a target for the action of neuroactive steroids. Brain Res Brain Res Rev 2005; 48(2): 328–38.
9. Melcangi RC, Giatti S, Pesaresi M, Calabrese D, Mitro N, Caruso D, García-Segura LM. Role of neuroactive steroids in the peripheral nervous system. Front Endocrinol 2011; 2: 104.
10. Milinković N, Ignjatović S, Šumarac Z, Majkić-Singh N. Uncertainty of measurement in laboratory medicine. J Med Biochem 2018; 37: 279–88.
11. Giatti S, Romano S, Pesaresi M, Cermenati G, Mitro N, Caruso D, Tetel MJ, Garcia-Segura LM, Melcangi RC. Neuroactive steroids and the peripheral nervous system: an update. Steroids 2015; 103: 23–50.
12. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, Noll J, Boring AM. Sex differences in brain maturation during childhood and adolescence. Cereb Cortex 2001; 11(6): 552–7.
13. Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, Blumenthal JD, Lerch J, Zijdenbos AP, Evans AC, Thompson PM, Giedd JN. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. Neuroimage 2007; 36(4): 1065–75.
14. Perrin JS, Hervé PY, Leonard G, Perron M, Pike GB, Piltot A, Richer L, Veillette S, Pausova Z, Paus T. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J Neurosci 2008; 28(38): 9519–24.
15. Gu YD. Diagnosis and treatment of injuries and disorders of the brachial plexus, 2nd ed. Shanghai: Fudan University Press, 2001. pp. 287–96.
16. Caruso D, Scurati S, Roglio I, Nobby L, Schenone A, Melcangi RC. Neuroactive steroid levels in a transgenic rat model of CMT1A Neuropathy. J Mol Neurosci 2008; 34(3): 249–53.

17. Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Caruso D, Melcangi RC. Sex differences in neuroactive steroid levels in the nervous system of diabetic and non-diabetic rats. Horm Behav 2010; 57(1): 46–55.

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