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Serum GD1a Ganglioside in Patients with Multiple Sclerosis

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In order to obtain more information concerning the neuronal damage in early multiple sclerosis(MS), the relative distribution of GD1a, one of the major human brain neuronal ganglioside fraction, was determined in the serum of patients with MS and of healthy subjects. The MS patients were with primary progressive MS (PPMS) and with remitting-relapsing MS during the first attack of the disease (FARRMS). An increase of GD1a portion in the serum of patients with PPMS and FARRMS was observed. The difference in relative proportion of GD1a between healthy subjects, patients with PPMS and FARRMS was statistically significant . It could be suggested that these findings are connected with the neuronal injury in the early phases of MS pathogenesis.They further support the concept of MS as a neuronal disease.

Key words: multiple sclerosis; ganglioside GD1a; serum.

Introduction

Multiple sclerosis (MS) is considered to be prototype of primary demyelinating disease in the central nervous system (CNS) in every textbook of neurology. However, in the past few years a considerable body of evidence indicates that neurons are also targets of the disease process [3, 4, 7, 9, 10, 15, 16]. Furthermore, in 2000 the Editorial paper of W a x m a n [17] in Archives of Neurology was entitled "Multiple Sclerosis as a Neuronal Disease". Although there is an increasing agreement that axonal loss is a major factor contributing to disability in the later stages of MS [2, 11, 13] the relation of neuronal damage to the pathogenesis of MS in the early stages of the disease remain to be elucidated.

Recent studies have shown axonal damage early in the evolution of MS [5, 13, 21]. The role of axonal degeneration in MS suggests that neuronal or axonal markers could be used to monitor disease progression.

Ganglioside GD1a is one of the major gangliosides in human brain neurons [19]. It was reported that GD1a was also the predominant ganglioside fraction in the bovine brain axons (without axolemma) [6]. The ganglioside spectra of normal blood plasma are remarkably stable and show only minor variations in samples from healthy donors of different age and sex [12]. However, in pathological conditions the plasma ganglioside spectrum may undergo pronounce changes [1, 14].

Recently, we first reported evidence that the serum ganglioside pattern undergoes significant changes in remitting-relapsing multiple sclerosis (RRMS) [20]. In order to obtain more information concerning the neuronal damage in early MS in this study the relative distribution of GD1a was determined in the serum of patients with primary progressive MS (PPMS) and of RRMS patients during their first MS attack (FARRMS).

Materials and Methods

Serum Samples

Sera were obtained from 20 patients with clinically definite MS and from 20 healthy subjects. Eight patients were evaluated during their first attack of the dis-

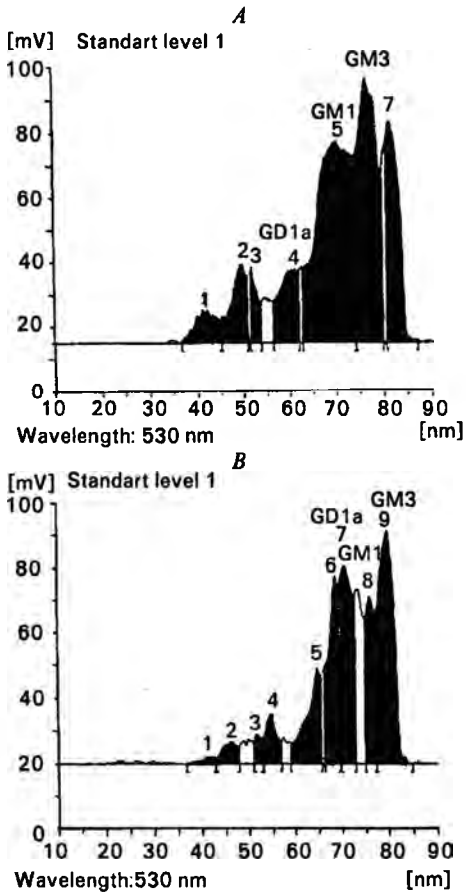

Fig. 1. Densitograms of Serum Gangliosides of MS Patients and Healthy Subjects
A — Healthy Subjects; *B* — Patients with first RRMS attack.(FARRMS)

ease of what later was definitely diagnosed as RRMS and 12 patients were with primary progressive MS (PPMS).

Isolation of serum gangliosides was performed by the method of I l i n o v et al. [8]. It includes the following stages:

a) dehydration of the sample by azeotropic distillation of the mixture of serum water/n-propanol = 1:10 (v/v);

b) total lipid triple extraction with cyclohexane (I), chloroform : methanol =1:1 (v/v) (II), and chloroform : methanol = 1:2 (v/v) (III);

c) non-polar lipids removal by preparative TLC with a mobile phase: chloroform : methanol: 0,3 % $CaCl_2$= 30:18:4 (v/v/v);

d) elimination of the blood sugar by Sep Pak technique according to W i l l i a m s and M c C l u e r [18].

e) HPTLC of the ganglioside fractions with a mobile phase: chloroform : methanol : 0,1 M sodium lactate = 55:40:10 (v/v/v).

The spots were visualized by spraying with orcinol reagent followed by local heating at 110°C and the gangliosides were quantified densitometrically. Bovine brain gangliosides (Calbiochem) were used as a test mixture for identification. Four independent analyses and quantifications were conducted for each MS group and healthy subjects.

The relative distribution of three serum gangliosides (GM3, GM1 and GD1a) in the serum of patients with RRMS during their first MS attack (FARRMS), of patients with primary progressive MS (PPMS) and of healthy subjects (HS) was recalculated on the basis of densitograms (Fig. 1 — *A* and *B*).

The Student's test was used to determine statistical differences between the groups using *P* value of less then 0,05 as the level of confidence. The data are presented as a mean value (M) ± standard error of mean (SEM) (Table 2).

Results

An increase of GD1a portion in the serum of PPMS and FARRMS was observed (Fig. 2 and Table 1).

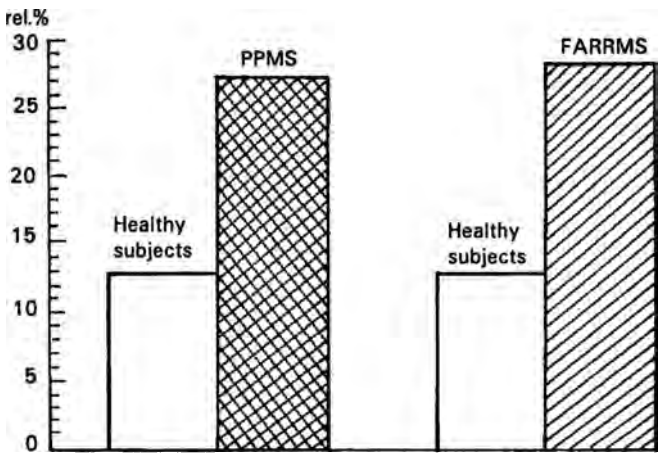

Fig. 2. Serum GD1a Gangliosides (in rel.%) in patients with PPMS, FARRMS and of Healthy Subjects

The difference in relative proportion of GD1a between healthy subjects patients with PPMS and FARRMS was statistically significant ($p<0.05$) (Table 2).

T a b l e 1. Relative Percentage of Serum GD1a Ganglioside Fractions in Healthy Subjects and in MS Patients

Group*	M ± SEM
HS (n = 20)	12.9 ± 0.40
FARRMS (n = 8)	28.3 ± 0.30
PPMS (n = 12)	27.3 ± 0.18

*HS — healthy subjects; FARRMS — patients with first RRMS attack; PPMS — patients with primary progressive MS; SEM — standard error of mean; n — number of evaluated subjects

T a b l e 2. Results of Student's Test Indicating Significance of Differences between HS, FARRMS and PPMS Patients with P Value of less than 0.05 Considered Significant

Group*	P value
HS vs FARRMS	0.02
HS vs PPMS	0.009

*HS — healthy subjects; FARRMS — patients with first RRMS attack; PPMS — patients with primary progressive MS

Discussion

This study has revealed a considerable increase of GD1a portion in the serum of PPMS and FARRMS patients. A statistically significant difference of GD1a portion was found between the healthy subjects and the patients with PPMS and FARRMS. These findings are in full concordance with our previous data concerning serum ganglioside spectrum in patients with RRMS [20]. The group of RRMS patients with their first MS attack had significantly rise of GD1a portion in the serum. The increase of serum GD1a in patients with PPMS was demonstrated for the first time in this study.

Early neuronal damage in patients with MS has been observed in vivo by magnetic resonance spectroscopy (MRS) which shows decreased levels of the neuronal specific marker N-acetylaspartate (NAA) in early stages of MS [5]. By assessing central brain NAA in MS patients with a wide range of disability and disease duration D e S t e f a n o et al. [5] showed that diffuse cerebral axonal damage begins in the early stage of RRMS and develops more rapidly in the earlier clinical stage of the disease.

Pathological studies of F e r g u s o n et al. [7] and Trapp et al. [15] applying modern morphological techniques, have provided evidence of axonal injury throughout active MS lesions. F e r g u s o n et al. [7] used amyloid precursor protein as a histopathological marker of damage axons, while T r a p p et al . [15] used confocal microscopy and immunohistochemistry applying an antibody to non-phosporylated neurofilament epitopes, which are increased in demyelinating axons.

Our recent electronmicroscopic investigations revealed early axonal damage in chronic relapsing experimental allergic encephalomyelitis which shares histopathological and immunological parameters with MS [21].

Considering the data mentioned above, and the fact that GD1a is one of the major ganglioside in human brain neurons, we could suggest that the increase of GD1a portion in the serum of FARRMS and PPMS is connected with the neuronal injury in the early phases of MS pathogenesis. Therefore, serum GD1a gangliosides could be used as neuronal markers to monitor disease activity. Our findings further

support the concept of MS as a neuronal disease. The neuronal pathology in the early MS argue for the early treatment of MS with agents directed toward neuronal protection.

References

1. Ayabe, M., S. Shichijo, M. Yokoyama. Diagnostic value of ganglioside patterns in plasma of human diseases. — J. Clin.Lab. Analysis, 3, 1989, 301-306.
2. Barnes, D., P. M. G. Munro, B. D. Youl, J. W. Prineas, W. I. McDonald. The longstanding MS lesion: a quantitative MRI and electron microscopic study. — Brain, **114**, 1991, 1271-1280.
3. Bjartmar, C., X. Yin, B. D. Trapp. Axonal pathology in myelin disorders. — J. Neurocytol., **28**, 1999, 383-395.
4. Compston, A., A. Coles. Multiple sclerosis. — Lancet, **359**, 2002, 1221-1231.
5. De Stefano, N., S. Narayanan, G. S. Francis, R. Arnaoutelis, M. C. Tartaglia, J. P. Antel, P. M. Matthews, D. L. Arnold. Evidence of axonal damage in the early stages of multiple scerosis and its relevance to disability. — Arch. Neurol., **58**, 2001, 65-70.
6. De Vries, G., W. Norton. The lipid composition of axons from bovine brain. — J. Neurochem., **22**, 1974, 259-264.
7. Ferguson, B., M. K. Matyszak, M. M. Esiri, V. H. Perry. Axonal damage in acute multiple sclerosis lesions. — Brain, **120**, 1997, 393-399.
8. Ilinov, P., D. Deleva, S. Dimov, E. Zaprianova. A variant for isolation of serum gangliosides. — J. Liquid Chrom. Rel. Technol., **20**, 1997, (8), 1149-1157.
9. Kornek, B., H. Lassman. Axonal pathology in multiple sclerosis: a historical note. — Brain Pathol., **9**, 1999, 651-656.
10. Lovas, G., N. Szilagyi, K. Majtenyi, M. Palcovits, S. Komoly. Axonal changes in chronic demyelinated cervical spinal cord plaques. — Brain, **123**, 2000, 308-317.
11. Scoldihg, N., R. Franklin. Axon loss in multiple sclerosis. — Lancet, **352**, 1998, 340-341.
12. Senn,H. J., M. Orth, E. Fitzke, H. Wieland, W. Gerok. Gangliosides in normal human serum. Concentration, pattern and transport by lipoproteins. — Eur. J. Biochem., **181**, 1989, 657-662.
13. Silber, E., M. K. Sharief. Axonal degeneration in the pathogenesis of multiple sclerosis. — J. Neurol. Sci., **170**, 1999, 11-18.
14. Suzuki, K. Gangliosides and disease: a review. — Adv. Exper. Med. Biol., **174**, 1985,407-418.
15. Trapp, B. D., J. Peterson, R. M. Ransohoff, R. Rudick, S. Mork, L. Bo. Axonal transection in the lesions of multiple sclerosis. — N. Engl. J. Med., **338**, 1998, 278-285.
16. Trapp, B. D., R. Ransohoff, R. Rudick. Axonal pathology in multiple sclerosis:relationship to neurologic disability. — Curr. Opin. Neurol., **12**, 1999, 295-302.
17. Waxman, S. G. Multiple sclerosis as a neuronal disease. — Arch. Neurol., **57**, 2000, 22-24.
18. Williams, A., R. McCluer. The use of Sep-Pak C18 cartidges during the isolation of gangliosides. — J. Neurochem., **35**, 1980, 266-270.
19. Yu, R., K. Iqbal. Sialosylgalactosyl ceramide as a specific marker for human myelin and oligodendroglial perikarya: gangliosides of human myelin, oligodendroglia and neurons. — J. Neurochem., **32**, 1979, 293-300.
20. Zaprianova, E., D. Deleva, P. Ilinov, E. Sultanov, A. Filchev, L. Christova, B. Sultanov. Serum ganglioside patterns in multiple sclerosis. — Neurochem. Research, **26**, 2001, (2), 95-100.
21. Zaprianova, E., O. Sotnikov, S. Sergeyeva, D. Deleva, A. Filchev, B. Sultanov. Axonal reaction precedes demyelination in experimental models of multiple sclerosis. — Morphology (in Russian), **5**, 2002,54-59.