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Serum Ganglioside GT1b Changes in Patients with Multilpe Sclerosis

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The relative distribution of ganglioside GT1b was determined in the serum of 52 patients with relapsing-remitting multiple sclerosis (RRMS) during the different stages of the disease and of 30 healthy subjects. There was statistically significant decrease of serum GT1b during the first attack of RRMS when axonal damage and demyelination are present in the central nervous system (CNS). In remission with a long duration, characterized by the occurrence of remyelination, serum GT1b increases twice in comparison to healthy individuals. These findings further support the concept concerning the role of GT1b in mediating the interactions between axons and oligodendrocytes needful for the formation of the myelin sheath and the maintenance of its integrity. Therefore, serum GT1b could be monitored as markers of demyelination and remyelination in the CNS of MS patients.

Key words: ganglioside GT1b, multiple sclerosis, serum, axon-oligodendrocytes interactions.

Introduction

Gangliosides, the most abundant sialyted glycoconjugates in the nervous system, are major cell surface determinants. The brains of higher vertebrates contain at least four major gangliosides: GM1, GD1a, GD1b and GT1b. They occur most prominently in the neuron where they comprise the major type of sialoconjugate in the plasma membrane. Gangliosides are present also in non-cell-associated form in blood, lymph, saliva and other body fluids. The main gangliosides in human blood serum are GM3, GM1, GD1a, GD1b and GT1b.

Gangliosides have been proposed to regulate cellular function including neuronal cell adhesion, transmembrane signalling and cell growth and differentiation. Perhaps the most dramatic interactions between neurons and glia in the central nervous system (CNS) occurs during myelination when oligodendroglial processes must recognize, adhere to, and ensheathe axons. A binding protein specific for neuronal ganglioside GT1b was detected on rat oligodendroglial membrane and it was suggested that GT1b play a role in mediating the interactions between axons and oligo-

dendroglia needful for myelination and maintenance the integrity of myelin sheath [11]. We recently first reported a significant increase of relative portion of GT1b in the brain and in the serum of Lewis rats during myelination [6]. There was an apparent correlation between the GT1b levels in the brain and in the serum during the different periods of myelination.

The disturbance of axon-oligodendroglial interrelationship occurs during demyelination and destruction of neuronal perikaryon, axons and oligodendrocytes. Multiple sclerosis (MS) is a demyelinating disease of the CNS with a considerable social impact. It is the major cause of non-traumatic disability in young adults. Traditionally, it was belived that MS was a primary demyelinating disorder and that neuronal and axonal damage occurred in chronic lesions. However, recent imaging and morphological studies indicate that neuronal loss and axonal injury are hallmarks of early MS [2, 10, 13, 15]. Considerable changes of serum gangliosides in pathological conditions [3, 16] were observed. We demonstrated a significant changes of GM1, GD1a and GM3 gangliosides in the serum of patients with early MS [16].

There is no data concerning serum GT1b variations in MS patients. In this study, the relative distribution of GT1b was determined in the serum of patients with relapsing-remitting multiple sclerosis (RRMS) during their first MS attack and in remission with a long duration.

Materials and Methods

Sera were obtained from 52 patients hospitalized at the Specialized Hospital for Active Treatment in Neurology and Psychiatry St. Naum, Sofia, with clinically definite MS according to Poser's criteria [9] and from 30 healthy subjects. Seven patients were evaluated during their first attack of the disease of what later was definitely diagnosed as RRMS, forty five patients were in remission with a long duration.

Isolation of serum gangliosides was performed by the method of I l i n o v et al. [5]. 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 % CaCl2= 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 [14]; 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. The Student's test was used to determine statistical differences between the groups using $P<0.05$ as the level of confidence.

Results

The relative percentage of the five major serum gangliosides (GM3, GM1, GD1a, GD1b and GT1b) during different stages of MS and in healthy subjects was recalculated on the basis of the densitograms (Fig. 1). The relative proportion of GT1b decreases from 6.19 % in the healthy subjects to 5.10 % during the first attack of MS. In remission with a long duration ganglioside GT1b content increases twice in compari-

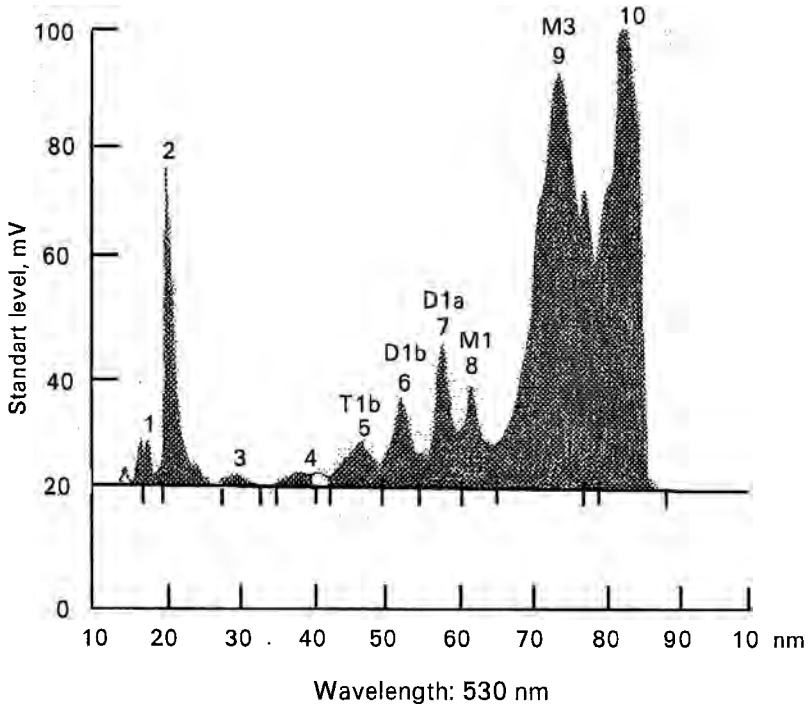

Fig. 1. Densitogram of serum gangliosides of a RRMS patient with a first attack of the disease

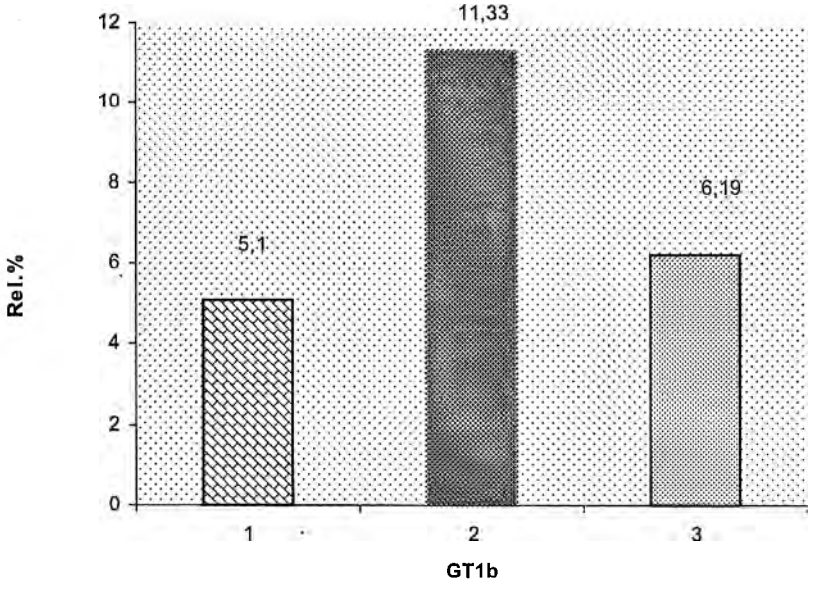

Fig. 2. Diagram of serum ganglioside GT1b of RRMS patients with a first attack of the disease (1), RRMS patients in remission with a long duration (2) and in healthy subjects (3)

T a b l e 1. Relative Percentage of Major Serum Gangliosides in Patients during Different Stages of Relapsing-Remitting MS and in Healthy Subjects

Gangliosides	I group (n=7) M ± SEM	II group (n=45) M ± SEM	III group (n=30) M ± SEM
GT1b	**5.10 ± 1.84**	**11.33 ± 2.04**	**6.19 ± 1.08**

M — mean value; SEM — standard error of mean; I group — RRMS patients with first attack of the disease; II group — RRMS patients in remission with a long duration; III group — healthy subjects;

son to its content in healthy subjects (Fig. 2). The relative portion of GT1b content during different stages of MS and in healthy subjects was statistically significant (P<0.05) (Table 1).

Discussion

In this study the content of ganglioside GT1b was analyzed in the serum of patients with RRMS during different stages of the disease. The results demonstrated in comparison to healthy individuals a statistically significant decrease of serum GT1b during the first attack of RRMS and a twice increase in remission with a long duration.

We could suggest that the decrease of serum GT1b in patients with early MS is connected with the disturbance of axon-oligodendroglia relationships due to axonal damage and demyelination, well demonstrated by the imaging and morphological studies of recent years [1, 2, 4, 7, 12]. In remission with a long duration remyelination may occur in MS [8]. The interactions between axon and oligodendrocytes had to be restored. Since ganglioside GT1b plays a role in mediating these interactions the twice increase of GT1b in the serum of RRMS patients does not seem to be surprising. This finding corresponds well with our previous studies concerning GT1b changes in the brain of Lewis rats with chronic relapsing experimental allergic encephalomyelitis (CREAE) during the early stages of the disease [17]. A significant decrease of relative portion of GT1b was revealed in the brain during the first clinical episode of CREAE, an animal model of RRMS.

In conclusion, the results of this study provide for the first time evidence that serum ganglioside GT1b undergoes statistically significant changes in RRMS patients during their first MS attack and in remission with a long duration. This finding further support the concept concerning the role of GT1b in mediating the interactions between axon and oligodendrocytes, needful for the formation of the myelin sheath and the maintenance of its integrity. Therefore, serum GT1b ganglioside could be monitored as markers of demyelination and remyelination in the CNS of patients with multiple sclerosis.

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