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GT1b Ganglioside Changes in Lewis Rat Serum During Myelination

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Ganglioside GT1b was suggested to play a role in mediating the interactions between axons and oligodendrocytes, needful for myelination and maintenance the integrity of myelin sheath. In this study, the relative distribution of GT1b was determined in Lewis rats serum during the different brain myelination periods. A high level of GT1b was detected in the serum just before the onset of brain myelination and of the time of active myelination compared to the GT1b level after the completion of myelination. This finding further support the concept concerning the role of GT1b in mediating the interactions between axons and oligodendrocytes. An apparent correlation was observed between the GT1b levels in the rat brain during the different periods of its myelination (determined in our previous studies) and the GT1b levels in the serum. Therefore, serum GT1b gangliosides could be monitored as markers of myelination and remyelination in the brain.

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

Introduction

Gangliosides are a family of sialic acid-containing glycosphingolipids highly enriched in the nervous system [19]. Trey are the major sialoconjugates in the brain. Gangliosides are mainly located on the surface of plasma membranes of neurons [9], glial cells [22] and in myelin of the central nervous system (CNS) [1]. The most abundant gangliosides in the adult mammalian brain are GM1, GD1a, GD1b and GT1b. They undergo characteristic changes in content and composition during development [18] and therefore could be used as markers for the different phases of brain maturation (synaptogenesis, myelination). We first reported [3, 4] significant ganglioside changes in rat and mouse medulla oblongata during the different periods of myelination. In the rat brain in the premyelination period and at the period of active myelination, the predominant ganglioside fraction was GT1b. Ganglioside GT1b was suggested to play a role in mediating the interactions between oligodendroglia and axons [15] needful for myelination and maintenance the integrity of myelin sheath.

Gangliosides occur also in non-cell-associated forms in blood, lymph, saliva and other body fluids. Changes in gangliosides in certain pathologies are reflected by their composition and blood serum levels [6, 7, 11, 14, 25, 26]. Therefore, information on blood serum gangliosides in normal and pathological states is of considerable importance [5].

There are no data concerning the serum ganglioside changes during brain myelination. In the present study the relative distribution of GT1b was determined in the sera of Lewis rats during the different period of myelination, characterized morphologically and histochemically by us in previous investigations [23].

Materials and Methods

Serum Samples

Sera were obtained from fifteen 9-day-old Lewis rats (the period of premyelination) (I group), fifteen 18-day-old Lewis rats (the period of active myelination) (II group) and from twenty-five 3-months old Lewis rats (the period after completion of myelination) (III group).

The relative distribution of four gangliosides (GM3, GM1, GD1a and GT1b) in the serum of Lewis rats of the three animal groups was recalculated on the basis of densitograms.

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; chloroform:methanol =1:1 (v/v) and chloroform:methanol = 1:2 (v/v);

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 [20];

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) and GM3 ganglioside (Sigma) were used as a test mixture for identification. Four independed analysis and quantification were conducted for each experimental group.

The relative distribution of five serum gangliosides (GM3, GM1, GD1a, GD1b and GT1b) in the serum of Lewis rats during the premyelination period, the period of active myelination and the period after completion of myelination was recalculated on the basis of densitograms (Fig. 1).

The Student's t-test was used to determine statistical differences between the groups using P value of less than 0,05 as the level of confidence.

Results

During the myelination of Lewis rats brain the relative proportion of GT1b in the serum increases from 14.30% at the premyelination period (I group) to 24.00% during the period of active myelination (II group). The relative content of GT1b decreases to 5.17% in the serum of Lewis rats after the completion of myelination (III group) (Fig. 2).

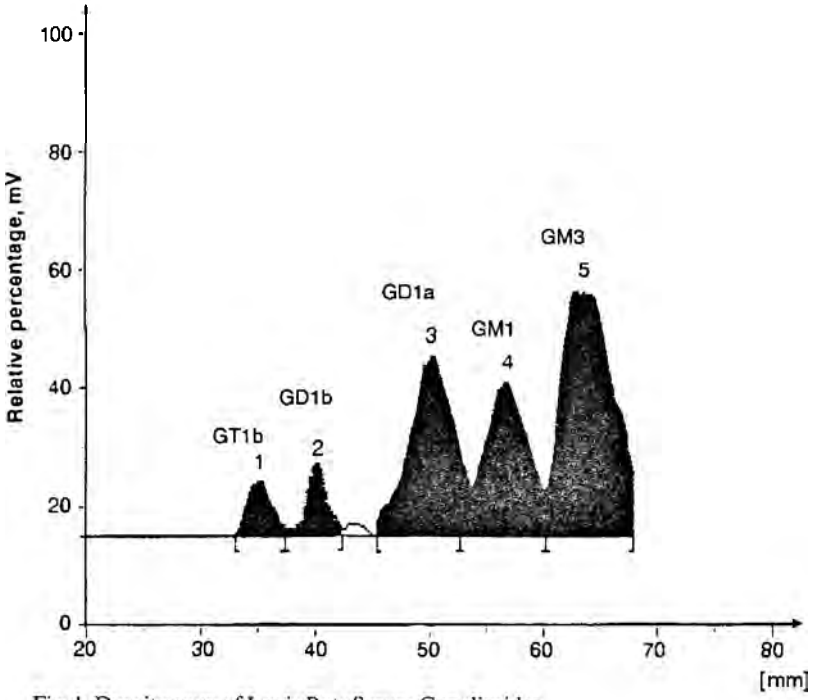

Fig. 1. Densitogram of Lewis Rats Serum Gangliosides

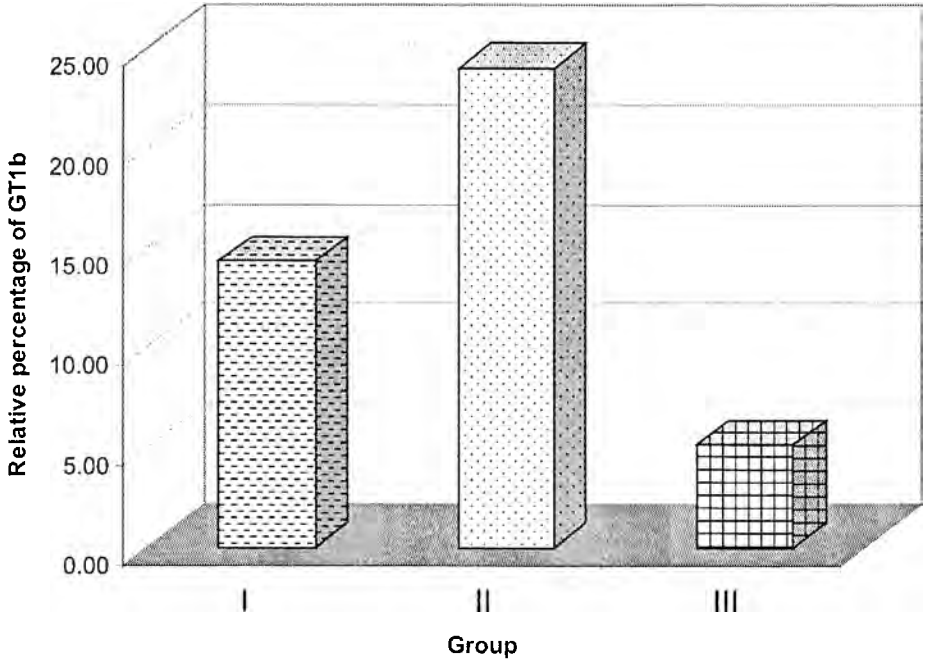

Fig. 2. Percentage Distribution of GT1b in the Serum of Lewis Rats during the Different Periods of Brain Myelination (in relative %) (I group — premyelination period; II group — period of active myelination; III group — period after completion of myelination)

The difference in relative proportion of GT1b between third group and the first and the second groups was statistically significant ($p < 0.05$) (Table 1).

Table 1. Relative Percentage of Major Gangliosides in the Serum of Lewis Rats during the Myelination in the Brain

Gangliosides	I group (n=15)	II group (n=15)	III group (n=25)
GT1b	14.30 ± 0.07	24.00 ± 0.05	5.17 ± 0.04
GD1b	42.20 ± 0.06	20.71 ± 0.04	6.94 ± 0.07
GD1a	2.90 ± 0.10	37.06 ± 0.06	19.07 ± 0.05
GM1	6.00 ± 0.08	5.14 ± 0.07	12.75 ± 0.04
GM3	34.20 ± 0.04	13.09 ± 0.08	56.07 ± 0.03

I group — premyelination period; II group — period of active myelination; III group — period after completion of myelination.

Discussion

The present study shows a high level of GT1b in the sera of Lewis rats before the onset of myelination and during the active myelination in the brain in comparison with the period of completed myelination.

The onset of myelination in rat brain is around the 10th postnatal day. For the beginning of this procees the myelinating oligodendrocyte must recognize the axon to be myelinated. The strategic location of gangliosides on the outer surface of the neural membranes, coupled with the great variability possible in the configuration of their oligosacharide chains makes them excellent candidates for selective intercellular recognition and/or adhesion molecules [10].

T i e m e y e r et al. [15] have reported that rat brain membrans contain a high-affinity structural specific protein for GT1b. They demonstrated that this receptor is found on central nervous system myelin and suggested that it may be positioned to mediate interactions between oligodendroglia and axons. On the other hand, Y a n g et al. [21] and C o l l i n s et al. [2] have proposed that GD1a and GT1b serve as complementary ligands for myelin-assosiated glycoprotein (MAG). MAG, a minor constituent of oligodendrocytes, is localized predominantly to the periaxonal glial plasmolemma [16]. Because of its periaxonal location, it is postulated that MAG may mediate axon-glial interactions [17]. The studes of S h e i k h et al. [13] on GM2/GD2 synthase knockout mice supported this hypothesis.

During the period of active myelination (17-20 postnatal days in the rat brain), when the compact myelin membrane is being formed, the interactions between oligodendrocytes and axons continue to be of great importance [24]. Since GT1b gangliosides play a role in mediating these interactions, the findings in our previuos studies [4] that GT1b fraction is predominant in the brain during premyelination period and during the active myelination, do not seem to be surprising. These results are in full concordance with the data presented here demonstating a high level of GT1b in serum of Lewis rats during the above mentioned myelination periods. There is an apparent correlation between the GT1b levels in the brain and in the serum during the myelination. The existence of such correlation is due to the fact that the blood-brain barrier in rats is not fully developed until postnatal day 24 [12]. Therefore, serum GT1b gangliosides could be used as markers of myelination, remyelination and demyelination (when the interactions between axons and oligodendroglia are disturbed). We recently first reported a significant decrease of relative portion of GT1b in the brain of Lewis rats with chronic relapsing experimental allergic encephalomyelitis, a demyelinating disease [27]. In the serum of these animals there was a significant decrease of GT1b (unpublished observations).

In conclusion, the data presented in this study clearly demonstrate for the first time that the content of GT1b in the serum of Lewis rats alter considerably during brain myelination. This finding further support the concept concerning the role of GT1b in mediating the interactions between oligodendroglia and axons, needful for myelination and maintenance the integrity of myelin sheath. Therefore, serum GT1b gangliosides could be monitored as markers of myelination and remyelination in the brain.

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