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Serum Ganglioside GM3 Changes in Patients with Early Multilpe Sclerosis

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Gangliosides GM3 are the major gangliosides in the endothelial cells forming blood-brain barrier (BBB) and in normal human blood serum. The relative distribution of GM3 was determined in the serum of patients with relapsing – remitting multiple sclerosis (RRMS) during the first attacks of the disease, when there was a significant BBB damage and of healthy subjects. A statistically significant decrease of serum GM3 in early RRMS was observed. These findings revealed for the first time a correlation between the decrease of serum GM3 during the first clinical signs of multiple sclerosis and the destruction of BBB. Therefore, serum gangliosides GM3 could be used as biomarkers of blood-brain barrier destruction.

Key words: ganglioside GM3, multiple sclerosis, serum, blood-brain barrier.

Introduction

Gangliosides (sialic acid – containing glycosphingolipids) are particularly abundant in the nervous system. Up to 95 % of cell gangliosides are present in the plasma membrane [10]. The major gangliosides in the endothelial cells forming blood- brain barrier (BBB) are GM3 (62 % of total gangliosides) [3, 6]. Gangliosides occur also in non-cell associated forms in blood plasma and other body fluids. The ganglioside spectra of normal blood plasma are remarkably stable, but show pronounced changes in pathological conditions [1, 2, 4, 8]. In multiple sclerosis (MS) significant changes of gangliosides GM1, GD1a and GT1b were revealed in the serum of patients during the first attacks of relapsing-remitting form of MS (RRMS) [12-15]. There are no data available on the serum GM3 level in MS patients. It was convincingly demonstrated that the damage of BBB occurred very early in the pathogenesis of MS [9].

The purpose of this study was to evaluate the serum level of GM3 in the patients with early MS when there was a significant BBB destruction. The relative distribution of GM3 was determined in the serum of patients with RRMS during their first attacks.

Materials and Methods

Sera were obtained from 7 patients with first attacks of MS of what later was definitely diagnosed as RRMS according to Poser's criteria [7] and from 30 healthy subjects.

Isolation of serum gangliosides was performed by the method of Ilinov 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 % $CaCl_2$= 30:18:4 (v/v/v); d) elimination of the blood sugar by Sep Pak technique according to Williams and McCluer [11]; 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 (GM1, GD1a, GD1b and GT1b) (Calbiochem) and GM3 ganglioside (Sigma) were used as a test for identification. The Student's test was used to determine statistical differences between the MS patients (I group) and healthy subjects (II group) using $P<0.05$ as the level of confidence.

Results

The relative percentage of GM3 gangliosides in patients with early MS and in healthy subjects was recalculated on the basis of the densitograms. The relative proportion of GM3 decreases from 69.10 % in the healthy subjects to 48.60 % during the first attacks of RRMS (Fig. 1). The relative portion of GM3 content during the first attacks of the disease and in healthy subjects was statistically significant ($P<0.05$) (Table 1).

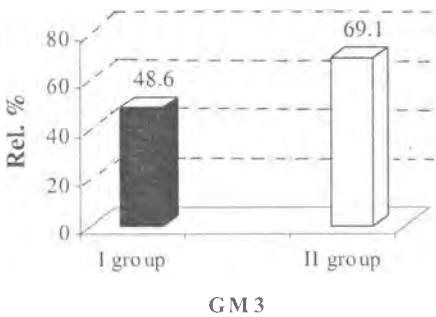

Fig. 1. Diagram of serum ganglioside GM3 of RRMS patients with first attacks of the disease (I group) and in healthy subjects (II group)

Table 1. Relative Percentage of GM3 Serum Gangliosides in RRMS Patients with First Attacks of the Disease and in Healthy Subjects

Ganglioside	I group (n=7) M ± SEM	II group (n=30) M ± SEM
GM3	48.60 ± 3.88	69.10 ± 0.45

M – mean value; SEM – standard error of mean; I group – RRMS patients with first attacks of the disease; II group – healthy subjects

Discussion

In this study the relative distribution of ganglioside GM3 was determined in the serum of patients with RRMS during their first attacks of the disease and in healthy subjects. The results demonstrated in comparison to healthy individuals a statistically considerable decrease of serum ganglioside GM3 in MS patients. The decrease of serum GM3 observed by us correlated with the significant destruction of BBB during the first attacks of MS, revealed by S h a r i e f et al. [9]. The endothelium of brain capillaries represents the structural basis for the BBB in vertebrates. D u v a r et al. [3] found that the major gangliosides in a new human cerebromicrovascular endothelial cell line are GM3. K a n d a et al. [6] established a method to yield sufficient quantities of highly purified human brain microvascular endothelial cells and compared their glycosphingolipid composition to that of human unbilical cord vein endothelial cells, as the representative of endothelial cells not forming BBB. They also detected that GM3 are major gangliosides of the BBB endothelial cells.

In conclusion, the findings of this study permits us to find, for the first time, that a considerable decrease of serum GM3 in early MS correlate with a severe destruction of blood-brain barrier. Therefore, we could suggest that serum ganglioside GM3 may be monitored as biomarkers of early damage of blood-brain barrier, which provides impetus to initiate early therapy.

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