Spinal Cord (2001) 39, 628–632

www.nature.com/sc

Original Article

Ganglioside patterns in human spinal cord

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Objective: To examine the distribution of gangliosides in human cervical and lumbar spinal cord.
Setting: Magdeburg, Germany.
Methods: The ganglioside distribution of human cervical and lumbar spinal cord enlargements from 10 neurological normal patients was analyzed. Gangliosides were isolated from different areas corresponding to the columna anterior, columna lateralis and columna posterior.
Results: Ganglioside GfD1b/GD1b and GD3 were the most abundant gangliosides in all examined tissues. The total concentration of sialic acid bound gangliosides GM2 and GM3 was less than 5%. The GD3 fraction constantly consisted of a double band as assessed by TLC after lipid extraction. There were significant differences in the ganglioside distribution when comparing tissue from the columna anterior, columna lateralis and columna posterior of the lumbar enlargement of the spinal cord.
Conclusion: Differences in the ganglioside composition in human spinal cord regions may reflect the different function of those molecules in the two regions investigated.
Spinal Cord (2001) **39,** 628–632

Keywords: gangliosides; spinal cord; human; glycosphingolipids

Introduction

Gangliosides are sialic acid-containing anionic amphiphiles located strategically in the cellular plasma membrane of vertebrates, most prominently in the central nervous system.[1,2] Gangliosides are involved in important, but as yet undefined functions in the physiology of the central nervous system whose concentration is maximal in synaptic regions of nerve cells.[3–6] Changes in ganglioside composition occur in the mammalian brain during development and aging.[7,8]

The pattern of glycosphingolipid content in the central nervous system varies between tissue types (white *vs* gray matter) and regions (ie cortex *vs* brain stem). Such differences in the distribution and content of gangliosides may provide information regarding the function of these lipid molecules.[3,5,9] Recent work has focused on determining the function of both endogenous and exogenous glycosphingolipids. Alterations in ganglioside composition occur in the central and peripheral nervous system in neurological disorders and several neuropathologies.[10–13] Gangliosides are also involved in autoimmune demyelination.[14] Schneider *et al* proposed a biological function of GM1 ganglioside in the damaged dopamine system of monkeys.[15]

Exogenously administered gangliosides promote neurite outgrowth *in vitro* and potentiate neurotrophic factor effects *in vitro* and *in vivo*.[16–20] Neuroprotective effects of gangliosides may arise from blockade of nitric oxide formation.[21,22] Interest in ganglioside function has been further heightened by clinical reports that indicate efficacy of monosialoganglioside (GM1) treatment for patients with spinal cord lesions and central nervous system (CNS) ischemia. GM1 therapy resulted in significantly improved functional recovery.[23–27]

Therefore, gangliosides are used as neuroprotective agents for the treatment of spinal cord injury[25] as well as ischemia and degenerative diseases.

There are few reports describing the ganglioside distribution in human spinal.[28,29] In this study, the distribution of gangliosides was examined in the gray matter of cervical and lumbar enlargement of the human spinal cord. Each region was further divided into regions that are functionally different (afferent *versus* efferent pathways). Increased knowledge of ganglioside distribution in the CNS may suggest functional evidence of the identified ganglioside species.

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Material and methods

Autopsy material
Human spinal cords were excised at autopsy within hours of death. Clinical records did not show any relevant neurological symptoms and disorders. The meninges were removed and the cords stored immediately at −20°C in acetone until analysis. All tissue analyses were performed between 1 to 12 weeks after removal and storage. Prior work indicates that the storage duration (12 weeks or less) does not affect ganglioside assays. Patients were excluded if the pathology record revealed any neuropathology after autopsy. The mean age of the sample group was 70 years (range 66–104). There were four male and six female tissue samples in the total autopsy material.

Ganglioside extraction
Gangliosides were extracted from spinal cord samples as follows. A 1 cm segment of spinal cord was taken from the cervical enlargement (C6–C7) and the lumbar enlargement (L2–L3). Each spinal cord sample was divided into three subregions corresponding to the columna anterior, columna lateralis and columna posterior areas, excluding the surrounding white matter. Tissue was cut with a freezing microtome into approximately 40 coronal sections throughout the sample.

The tissue slices for each area were collected and homogenized for 10 min at 4°C in acetone and incubated at 20°C for 15 min. After centrifugation, the supernatant was discarded and 4 ml of cold acetone was added. This procedure was repeated twice. The pellet was reextracted (three times) with 3.3 ml of chloroform-methanol-distilled water (1 : 2 : 0.3 v/v/v) and incubated for 15 min at 37°C in a water bath. The ratio of chloroform-methanol-distilled water was adjusted to 1 : 1 : 1 v/v/v.

This solution was shaken overnight and gangliosides were then separated from other lipids by partitioning into the aqueous methanol (upper) phase. The lower phase was removed with a syringe and discarded. The membrane between both phases was saved together with the remaining upper phase and dried under air in a 37°C water bath and then used for ganglioside analysis.[30–32]

Separation and analysis of gangliosides were carried out on thin layer chromatography (TLC) using precoated silica gel 60 high-performance-thin-layer chromatography (HPTLC) plates (0.025 × 10 × 20 cm; Merck). An aqueous solution containing 5 μg of sialic acid was applied to HPTLC plates followed by activation at 100°C for 30 min. The plate was developed with a chloroform-methanol-triethanol $MgCl_2$ buffer [2440 mg $MgCl_2$ and 746 mg triethanolamin in 1 liter of distilled H_2O] (60/35/8.2 v/v/v/) and dried. Bands were visualized by spraying with resorcinol-hydrochloric acid followed by heating the covered plate at 95°C for 30 min (see Figure 1).[32]

Ganglioside content was determined by direct densitometric scanning of the resorcinol-positive bands on HPTLC plates (Shimadzu High Speed Thin Layer Chromatography Scanner CS-920) using a sample wavelength of 560 nm with zigzag scan mode. A ganglioside mix was used as a standard to identify the ganglioside fractions. All data were expressed as percentage of total ganglioside bound-sialic acid. Ganglioside species that represented less than 5% of the total were not included in the data analyses. The following

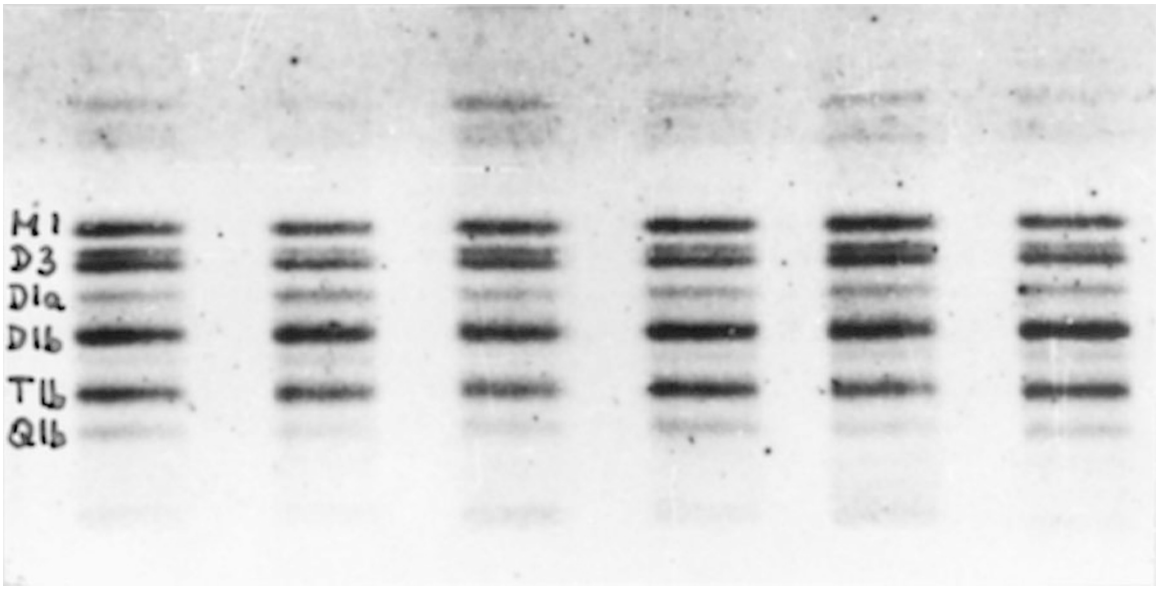

Figure 1 Representative TLC-plate for visualizing ganglioside content in different spinal cord regions of the lumbar enlargement. Lanes 1–3 are from the columna anterior, whereas lanes 4–6 represent ganglioside content from the columna posterior. The different ganglioside fractions are labeled on the left side (M1 = GM1, D3 = GD3 etc.)

fractions were examined and included into the data analysis: GQ1b, GT1b, GD1b (unidentified + GD1b), GD1a, GD3 (double band) and GM1. Due to sample loss during the process of separating the lower lipid phase (see Materials and methods section – meaning not also recovering the membrane between the aqueous phase and the lower phase with other lipids) the analysis for the subregions was restricted to the lumbar enlargement. Statistical analyses were done to compare the total ganglioside distribution between the lumbar and the cervical enlargement, as well as between the three subregions sampled (columna anterior, lateral and posterior) for the lumbar enlargement using the Wilcoxon matched-pairs test.

Results

No differences of total ganglioside bound sialic acid were found when comparing tissue obtained from male or female autopsy material. The percentage of distinct ganglioside fractions relative to total ganglioside-bound sialic acid obtained from 5 μg sialic acid in tissue taken from the cervical and lumbar enlargements are shown in Table 1. These data represent the ganglioside distribution from either the cervical or lumbar enlargement regardless of subregions (ie columna anterior, columna lateralis, columna posterior). Ganglioside GD1b (about 24% for the cervical enlargement and 26% for the lumbar enlargement) predominated. Furthermore, the distribution of gangliosides was characterized by a large proportion of GD3 and relative small amounts of Gd1a and GQ1b. Statistical analyses revealed differences comparing the ganglioside distributions among the cervical and lumbar enlargement of the ganglioside fraction GfD1b/GD1b and GQ1b.

Comparisons of different subregions (ie columna anterior, columna posterior and columna lateral) within the lumbar enlargement revealed no differences in the ganglioside fractions: GD3, GD1a, GD1b or GQ1b. However, when comparing ganglioside GM1 levels in the columna posterior to levels in the columna anterior and columna lateralis, its content was significantly lower ($P<0.05$). Conversely, GT1b levels were significantly higher in the columna posterior when compared to the columna anterior and columna lateralis ($P<0.05$) (Table 2).

Discussion

The distribution in mammalian spinal cord differs significantly from cerebral tissue.[2] For example ganglioside levels in the spinal cord are only 1/3 (white matter) to 1/10 (gray matter) of those levels found in cerebral tissue. Such large differences support the view that ganglioside levels and types may be correlated with function. The ganglioside distribution in human spinal cord is relatively unexplored.[2,12,29] The significant differences in this report indicate that ganglioside distribution may be critical for appropriate neuronal function.

These patterns of ganglioside distribution are in accordance to the findings by Svennerholm *et al*[29] but compared to more earlier reports by Ueno *et al*[2] data presented here show higher proportions of GD1b, GT1b and GQ1b. Accordingly higher proportions of GM1 were found. Methodological differences in the studies have to be considered since least soluble b-series gangliosides can be lost on the Sephadex column during isolation.[33]

Different molecular species of gangliosides occur in varying proportions in various vertebrate tissues and organs, with the highest concentrations being found in the gray matter of the mammalian nervous system.[6] Gangliosides in this region differ qualitatively from those in other tissues. Whereas gangliosides in the CNS are primarily of the ganglio-series, those of the peripheral nervous system and extraneural tissues contain higher levels of the lacto- and globo-series

Table 1 Ganglioside distribution in human cord spinal enlargements

Ganglioside fraction	*Cervical enlargement* n = *11*	*Lumbar enlargement* n = *18*
GM1	15.08 ± 0.82	14.16 ± 0.94
GD3 (double band, including unidentified band)	19.94 ± 0.76	18.98 ± 0.49
GD1a	11.27 ± 0.93	12.35 ± 0.76
GfD1b/GD1b	24.88 ± 0.69	26.68 ± 0.48*
GT1b	18.66 ± 0.72	19.14 ± 1.21
GQ1b	10.09 ± 0.41	8.27 ± 0.39*
		$P<0.05$

Ganglioside distribution of the cervical and lumbar enlargement of the human spinal cord. Values are expressed as percentage of total ganglioside-bound sialic acid (mean ± SE). *Statistically significant

Table 2 Ganglioside distribution of human spinal cord in subregions (lumbar enlargement)

Ganglioside fraction	*Columna anterior* n = *6*	*Columna lateralis* n = *6*	*Columna posterior* n = *6*
GM1	15.67 ± 0.8	15.21 ± 1.1	11.61 ± 1.1
			*$P<0.05$
GD3 (double band, including unidentified band)	19.38 ± 1.9	18.78 ± 1.4	18.79 ± 0.8
GD1a	11.57 ± 0.9	12.27 ± 0.3	13.23 ± 0.7
GfD1b/GD1b	27.01 ± 0.9	26.24 ± 0.6	26.78 ± 1.1
GT1b	17.72 ± 1.3	18.71 ± 1.2	21.00 ± 1.6
			*$P<0.05$
GQ1b	7.76 ± 0.8	8.61 ± 1.2	8.44 ± 1.1

Ganglioside distribution within subregions of human spinal cord lumbar enlargement. Values are expressed as percentage of total gangliosid-bound sialic acid (mean ± SE). *Statistically significant

gangliosides. Gray matter of the brain contains predominantly GM1, GD1a, GD1b, GT1b and GQ1b. GM1 and GD1a are found predominantly in white matter.[34]

Ganglioside analysis of human motor and sensory nerves revealed that ceramide composition of sensory nerve GD1a, GD1b, and GM1 differed apparently from those in the motor nerve.[35] Furthermore, motor nerve myelin contains GM1 (about 15% of total ganglioside), whereas sensory nerve myelin contains only trace amounts of GM1 (less than 5%), by TLC analysis.[36]

Dawson and Stefansson report that ganglioside GD1a exhibits a gradient in human spinal cord where its lowest concentrations are in the cervical region, increasing in the lumbar and then thoracic regions of the cord, reaching its highest level in the sacral region.[28] Other gangliosides did not show such a gradient. This gradient was not related to the amounts of myelin and neuronal tissues in the different cord regions.[37,38]

In this study, we find evidence for a possible relationship between cord function(s) in the posterior afferent region. In that region significantly different levels of these two gangliosides (GM1 [lower] and GT1b [higher]) were seen when compared to the medial cord region (substantially a 'control' area), and with the anterior region of the cord.

These significant differences in ganglioside patterns (GT1b [high] and GM1 [low]) seen in areas of the cord from which efferent (anterior) nerve trunk processes originate, pointing to a possible role of gangliosides associated with efferent function. In future work, we intend to explore changes in the ganglioside content in the human spinal cord and for various central nervous pathologies. Additionally, manipulating the ganglioside distribution in *in vitro* systems to achieve specific membrane properties, may allow us to identify functional consequences of alterations in the ganglioside distribution.

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