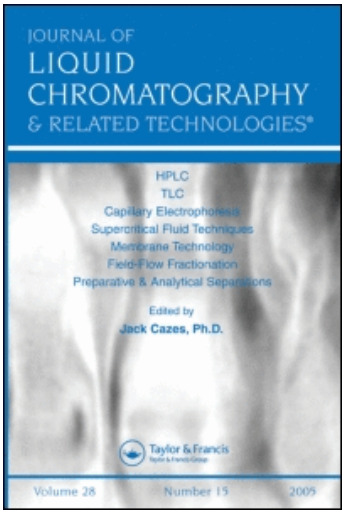

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparative Analysis of Brain and Omentum Tissue Gangliosides

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To cite this Article Vajdi, Mehran(1992) 'Comparative Analysis of Brain and Omentum Tissue Gangliosides', Journal of Liquid Chromatography & Related Technologies, 15: 17, 2959 — 2966
To link to this Article: DOI: 10.1080/10826079208016362
URL: http://dx.doi.org/10.1080/10826079208016362

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(17), 2959-2966 (1992)

COMPARATIVE ANALYSIS OF BRAIN AND OMENTUM TISSUE GANGLIOSIDES

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Abstract

Procedures for isolation and purification of individual gangliosides from bovine brain, porcine brain, bovine omentum and porcine omentum have been developed yielding high quality products with better than 98% purity.

Bovine and porcine brain gangliosides yielded different patterns and concentrations of individual gangliosides. Contrary to bovine brain gangliosides, porcine brain showed an additional band corresponding to an appreciable quantity of GD3.

The major difference in ganglioside composition was noted between brain and omentum. Significant variations in types and composition of individual gangliosides were observed in the omentum tissue. Presence of double and triple TLC bands indicating various fatty acids on the ganglioside molecules were characteristic features of the omentum tissue which were not observed in the brain gangliosides.

Introduction

Gangliosides are a group of glycolipids which contain one or more sialic acid moieties. Presence of complex gangliosides in brain is suggestive of a functional role in the central nervous system. Since gangliosides are also found outside the nervous system, they are considered to be an important part of the surface membrane of most cells of animals. Immuno-properties of

glycolipids and gangliosides are also important since they contribute to the immunological expression of cells and can be used in clarification and elucidation of immunobiological reactions taking place in certain disorders.

Lipids derived from omental tissues have shown to stimulate the production of capillary growth and have important implications in major diseases. This biological phenomena has significant therapeutic applications in clinical areas such as wound and bone fracture healing, burns, and most important, tissue and organ ischemia (1-4). Therefore, tissues available in large quantities that contain angiogenic activity provide opportunities for isolation, structural analysis and extensive animal and clinical studies.

Neutral glycosphingolipids and particularly gangliosides have recently been shown to be associated with a variety of cellular responses and to modulate the activity of several growth factor receptors (5). The potential role of gangliosides especially GM1 (6-8) as therapeutic agents have been recently investigated and shown to be valuable. Considering the ubiquity of distribution of these plasma membrane constituents, future research of gangliosides is necessary and continues to be important. Consequently, familiarity with gangliosides from various tissues has become evident and crucial. The purpose of this study was separate, purify and identify gangliosides of brain and omentum tissues.

Materials & Methods

Lipids from brain and omentum tissues were extracted with 20 volumes of chloroform/methanol (2:1). Gangliosides were separated from the extract by the Folch procedure (9) and cleaned according to an in house developed process. Reverse phase column chromatography was used to separate salt and proteinaceous

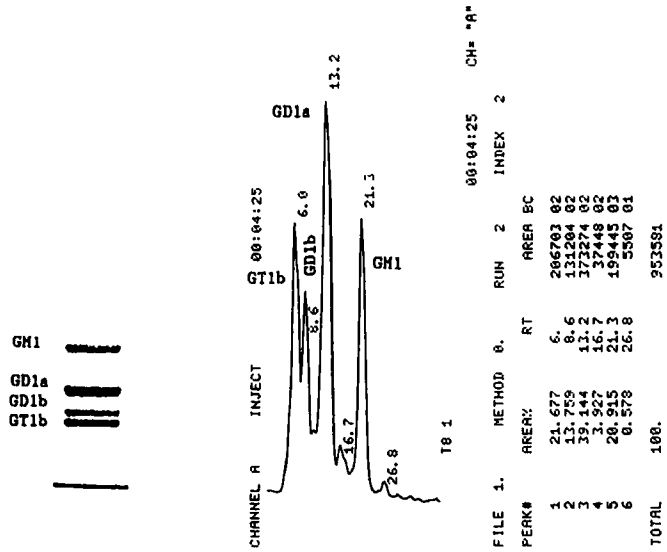

Figure 1. HPTLC and densitometric analysis of bovine brain gangliosides

materials. Gangliosides were removed from the column with methanol and evaporated for the TLC analysis. The samples were solubilized in choloroform/methanol (1:1) and applied to the HPTLC Silica plates (Merck) by the Camag Linomat IV applicator. The developing solvent consisted of C/M/.25% aqueous $CaCl_2$ (55/45/10). The plates were sprayed with 50% H2S04 solution and scanned with the Camag TLC scanning densitometer using white light reflactance for measurement.

Results and Discussion

HPTLC analysis of bovine brain gangliosides presented in Figure 1 shows four major gangliosides. According to the densitometric evaluation of the mixture, GD1a comprises 39% of the total gangliosides (Table 1). As shown in Figure 2, porcine brain ganglioside, on the other hand, consists of five major

TABLE 1. GANGLIOSIDES COMPOSITION OF BRAIN AND OMENTUM TISSUE

Relative Composition of Major Gangliosides (%)

	BOVINE BRAIN	PORCINE BRAIN	BOVINE OMENTUM	PORCINE OMENTUM
GM3	-	-	39.9	16.9
GM2	-	-	-	2.7
GM1	20.9	25.7	17.0	14.0
GD3	-	13.8	-	13.7
GD1a	39.1	31.9	16.2	38.3
GD1b	13.7	11.4	-	-
GT1b	21.7	16.2	-	8.4
GQ	-	-	-	1.0

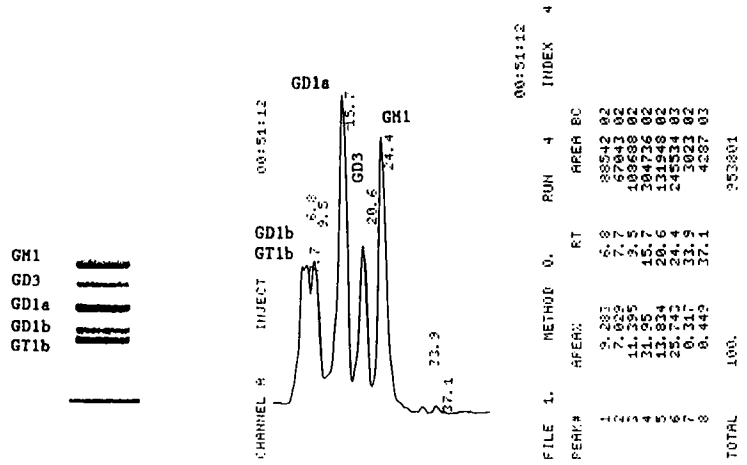

Figure 2. HPTLC and densitometric analysis of porcine brain gangliosides

gangliosides. Disialogangliosides, GD3, is unique here and is present in appreciable quantity. Densitometric quantification of the porcine brain gangliosides indicated 13.8% GD3 in the mixture (Table 1).

Omentum gangliosides are very unique with regard to the types and percent composition of the individual gangliosides. As shown in the bovine omentum (Figure 3), GM3 comprises 39.9% of the mixture (Table 1). Only three major gangliosides were observed in the bovine omentum mixture. As shown clearly in the densitometric spectrum of the mixture (Figure 3), GM3, GM1 and GD1a are present in doublets indicating presence of different fatty acid chains on the ganglioside molecules.

The Porcine omentum mixture, solublized in chloroform/menthanol (1:1) was colorless yielding clear TLC plate with good resolutions among the individual gangliosides. The

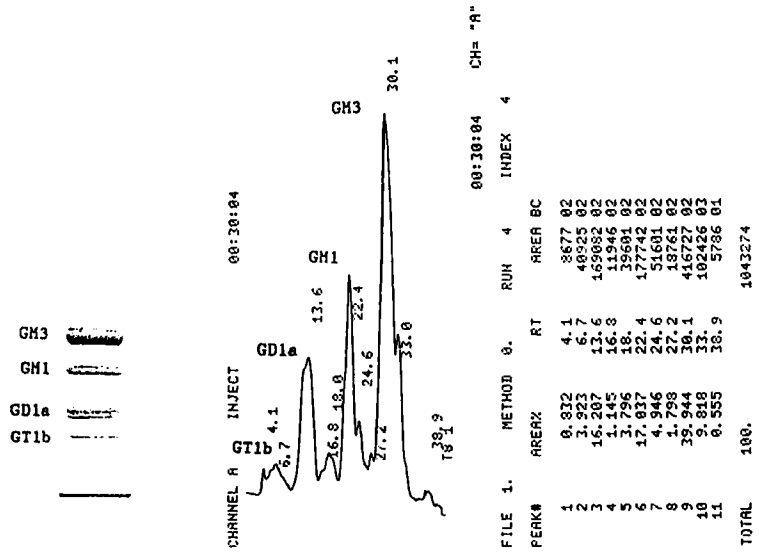

Figure 3. HPTLC and densitometric analysis of bovine omentum gangliosides

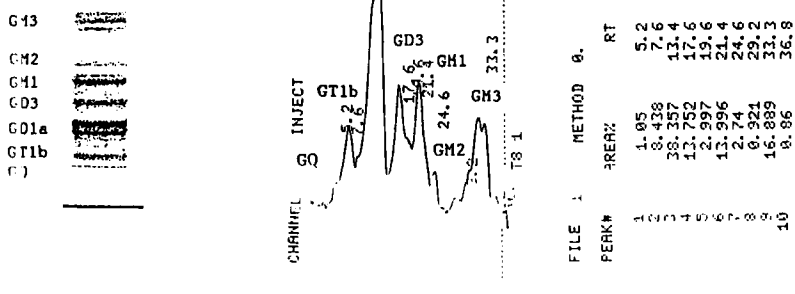

Figure 4. HPTLC and densitometric analysis of porcine omentum gangliosides

mixture consisted of seven gangliosides (Figure 4) with GD1a comprising 38.3% of the composition. Additional bands observed in the omentum mixture had RF values close to GQ and GM2 gangliosides. Distinct characteristics of the porcine omentum gangliosides are clearly observed in Figure 4 by the presence of doublets for GM1 and GD1a and triple bands for GM3 gangliosides. The appearance of additional bands for GM1, GD1a and GM3 maybe indicative of the presence of different fatty acid chains on the gangliosides molecules.

Characteristic differences in individual gangliosides were observed not only between the species but in brain and omentum tissue. The differences were found to be qualitative and quantitative in nature and unique.

Acknowledgement

This work was supported by Angio-Medical Corporation.

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