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Distribution of Gangliosides in Parenchymal and Non-parenchymal Cells of Rat Liver

Kunihiro Ueno, Yasunori Kushi, Chiaki Rokukawa and Shizuo Handa

(Department of Biochemistry, Faculty of Medicine, Tokyo Medical and Dental University, 5-45, Yushima 1 Chome, Bunkyo-ku, Tokyo 113, Japan)

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Summary Parenchymal and non-parenchymal cells were isolated from rat liver with purities of more than 90%. Total and ganglioside sialic acid contents were higher in non-parenchymal cells than in parenchymal cells. Thin-layer chromatography of gangliosides showed that the main component in rat liver was ganglioside GM3 and that this was abundant in non-parenchymal cells. Parenchymal cells had ganglioside GD1b as the main component and less GM3 than non-parenchymal cells. These results suggested that the main ganglioside of rat liver, GM3, arises mainly from non-parenchymal cells.

Introduction

Mammalian liver contains parenchymal cells or hepatocytes and non-parenchymal cells. The latter is a general term including sinusoidal endothelial cells, Kupffer cells and fat storing cells which are associated with the space of Disse. The results of biochemical studies on homogenized liver were often attributed to parenchymal cells because of their majority and little attention was paid to the contribution of non-parenchymal cells. A recent review reports that many enzyme activities have different distributions in the two cell types(1). A stereological study on rat liver showed that parenchymal cells and non-parenchymal cells

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Abbreviations: TLC, thin-layer chromatography; HEPES, N-(2-Hydroxyethyl)piperazine-N'-2-ethanesulfonic Acid. Abbreviations for gangliosides follow the nomenclature system of Svennerholm(26).

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accounted for 93% and 7% of the total liver cell volume, respectively, and that non-parenchymal cells contained 27% of the total plasma membrane by volume(2). These non-parenchymal cells are not negligible, especially with respect to substances that are localized on the cell surface membrane. The cell surface is enriched in sialic acid-containing compounds and the biological roles of these compounds have received great attention(3).

The object of the present study was to isolate and characterize parenchymal and non-parenchymal cells according to their content of sialic acid-containing lipids. Liver glycolipids have been well studied in human(4, 5), cow(6), rat(7, 8) and mouse (9), but to our knowledge there is no report on their distribution in isolated cells from normal rat liver.

Materials and Methods

Materials Male Wistar rats weighing approximately 200g were used. Normal human liver was obtained at autopsy from an adult patient who died of an extrahepatic disease. A normal adult human brain, which was fixed in formalin for 3 weeks was used for isolation of the standard ganglioside mixture from the cerebral white matter. Collagenase(EC 3.4.4.19) type 1A from Clostridium histolyticum was purchased from Sigma Chemical Co., St. Louis, Mo. The other reagents were of analytical grade.

Preparation of cells The procedure for isolating parenchymal and non-parenchymal cells was a modification of published methods(10-13). The abdominal cavity of the rat was opened and the inferior vena cava was canulated at just above the renal veins. Liver perfusion was started with 50ml of 10mM HEPES buffer, pH7.4, containing 100U of heparin. Then the portal vein was cut and the thoracic cavity opened and the inferior vena cava ligated. The perfusion was continued with 100ml HEPES buffer and 100ml HEPES buffer with 4.8mM calcium chloride and 50mg collagenase. The liver was removed from the rat and cut into small pieces. These were further digested in 50ml HEPES buffer with calcium chloride and collagenase at 37°C for 8min. with shaking. Total liver cells were obtained by filtration of the digested liver pieces through 3 layers of nylon mesh. The total liver cell suspension was centrifuged at 50xg for 2min. Parenchymal cells were obtained by 2 more washings of the precipitate.

Non-parenchymal cells were purified from the supernatant of the first centrifugation. The supernatant was spun down at 500xg for 10min. and the precipitate was treated with hypotonic saline twice similarly.

Preparation of gangliosides Gangliosides from liver, brain and isolated cells(17 to 520mg protein) were obtained by the published method(14) with slight modifications as follows. A large excess of ethyl acetate was added and the mixture was stirred at 37°C for 1h. to neutralize the alkaline stable lipid solution. Sephadex column chromatography for desalting was monitored with a LKB 2138 Uvicord S with a 206nm filter.

Thin-layer chromatography and densitometry Gangliosides were developed with 2 solvent systems, chloroform-methanol-water, 60:40:9(v/v/v), containing 0.02%(w/v) dihydrate calcium chloride, and chloroform-methanol-2.5N ammonia, 60:35:8(v/v/v), and detected by resorcinol-HCl reagent. The plate developed in the former solvent was used for densitometric quantification(15).
Preparation of total sialic acid Homogenates of the liver and isolated cells were hydrolyzed in 0.1N sulfuric acid at 80°C for 2h. and released sialic acids were separated on Dowex 1-X8(formate form) columns(16).
Other biochemical methods The protein contents of tissue or cell homogenates in 1% sodium dodecylsulfate was determined by the method of Lowry et al.(17) using crystalline bovine serum albumin as a standard. Sialic acid was determined by the resorcinol-HCl method(18). The activity of glucose-6-phosphatase(EC 3.1.3.9) was determined as a marker of parenchymal cells by the published method(19).
Light microscopy The purity of isolated cells was examined under a light microscope. Thin sections of embedded cells were stained with toluidine blue and photographed. Enlarged prints were made and pictures of each cell were counted differentially for the cell number at random.
Electron microscopy The purity and integrity of isolated cells was examined under a transmission electron microscope. The cell pellet about $1mm^3$ in size was fixed in 2.5% glutaraldehyde followed by 1% osmium tetroxide in 0.1M phosphate buffer, pH7.4. Thereafter, the sample was dehydrated in cold graded ethanol and embedded in EPON 812. The ultra-thin section was stained with uranyl acetate and lead citrate and examined under a Hitachi HU-12 electron microscope at an accelerating voltage of 75KV.

Results and Discussion

Cell isolation method The most common method for isolating liver cells is collagenase perfusion method from the portal vein to the inferior vena cava based on the method of Berry and Friend(10). We found liver perfusion from the inferior vena cava to the portal vein in reverse of *in vivo* circulation to be easier and more successful than the common method. Soon after we found the report by Crisp and Fogson(13) who used a similar reversed perfusion method. We also were able to obtain both parenchymal and non-parenchymal cells from a single rat liver.
Purity of isolated cells Light microscopic examination showed the parenchymal cell fraction had 5% contamination by non-parenchymal cells but the non-parenchymal cell fraction was almost free of contamination by parenchymal cells(Table I, Fig. 1).

The contamination of the parenchymal cell fraction may be due to incomplete separation of each cell type in the total cell

Table I Properties of human liver, rat liver, parenchymal cells (PC) and non-parenchymal cells(NPC).

	Protein	Purity	Glucose-6-phosphatase	Total sialic acid	Ganglioside sialic acid
Human liver	98 (1)			3.05 (1)	116 (1)
Rat liver	176±3(3)		3.0±0.6(2)	2.26±0.17(5)	105±25(4)
PC		95	22.2±0.1(2)	1.81±0.56(3)	140±16(3)
NPC		100	2.1±0.4(2)	2.35±0.28(3)	291± 6(2)

protein:mg/g wet wt.; purity:% by cell number; glucose-6-phosphatase:n moles/mg protein/min.; total sialic acid: μg/mg protein; ganglioside sialic acid:ng/mg protein. Values represent mean±S.D. Figures in parentheses are numbers of samples.

suspension and also due to the sticky nature of Kupffer cells(20). The glucose-6-phosphatase activity as the marker of parenchymal cells(13, 21) was low in the non-parenchymal cell fraction, amounting to one tenth the activity of the parenchymal cell fraction. As this enzyme was abundant in parenchymal cells and located solely in microsomes(19), a possible explanation of the enzyme activity in the non-parenchymal cell fraction is microsomal contamination derived from broken parenchymal cells as seen in Fig.

Total and ganglioside sialic acid contents Total sialic acid contents of rat liver varies in literature (7, 22). Our data were of the order of 2 to 3μg/mg protein in human and rat livers. In the isolated cell fractions, non-parenchymal cells had more than twice the ganglioside sialic acid contents of parenchymal cells. Since the sialic acid is concentrated in the plasma membrane(23), its high content in non-parenchymal cells may be in accord with the adhesive nature(20) and the fuzzy coat(24) of Kupffer cells.

Ganglioside distribution The ganglioside distribution pattern on TLC and the densitometric data are shown in Fig. 2 and Table II. Each component of the rat liver gangliosides was identified not only by its mobilities in 2 different solvent systems but also by structural analysis on isolated components, including carbohydrate analysis, sialidase(EC 3.2.1.18) treatment, methylation analysis and direct inlet mass spectrometry of permetylated gangliosides (data not shown). The standard brain sample had a different

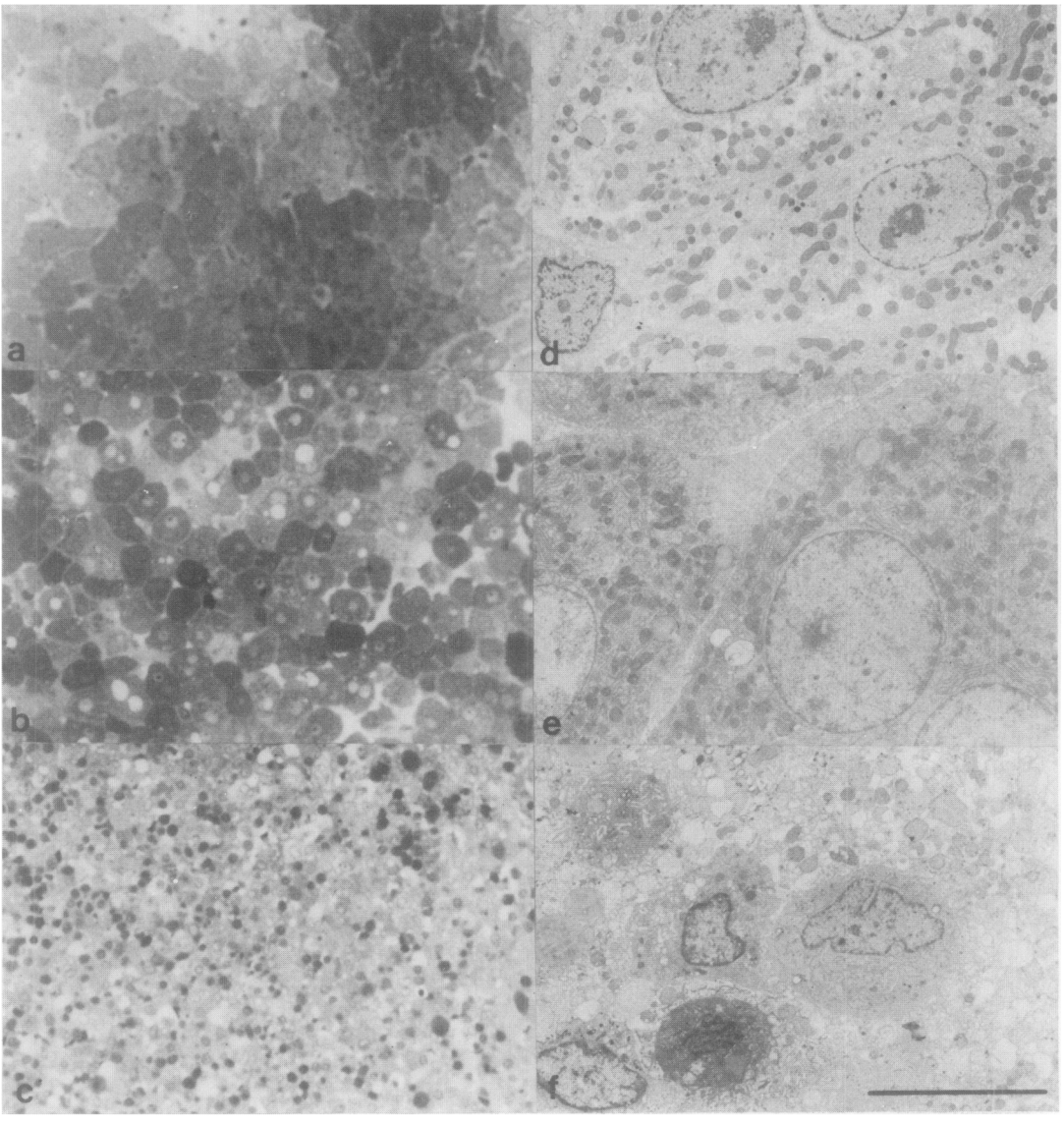

Fig. 1 Light microscopic pictures of rat liver(a), parenchymal cells(b) and non-parenchymal cells(c) (X320), and electron microscopic pictures of rat liver(d), parenchymal cells(e) and non-parenchymal cells(f) (X2400; bar, 10μm)

distribution pattern(Fig. 2, lane 1) from the results for brain stored in a freezer(14, 15), probably due to degradation of polysialo-gangliosides by formalin fixation. The distribution of human liver gangliosides was close to other reported results(4). Rat liver showed a different distribution from that of human liver, showing low G_{M3} and high polysialo-gangliosides contents.

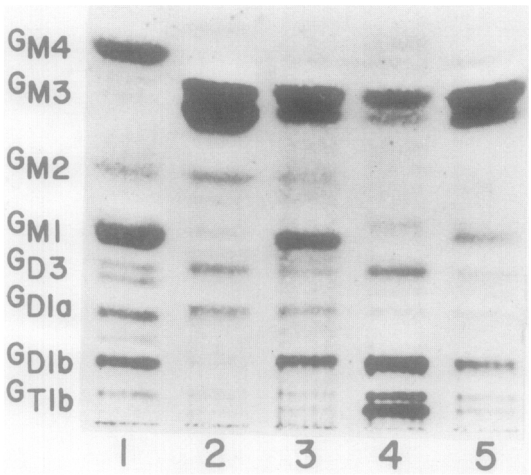

Fig. 2 TLC of gangliosides from formalin-fixed human cerebral white matter (1), human liver (2), rat liver (3), parenchymal cells (4) and non-parenchymal cells (5). Samples (2 to 3.5 μg sialic acid) were applied on the TLC plate and developed in chloroform-methanol-water, 60:40:9 (v/v/v), containing 0.2% (w/v) dihydrate calcium chloride. Spots were detected by spraying with resorcinol-HCl reagent.

Parenchymal and non-parenchymal cells showed different distribution patterns. In parenchymal cells, G_{M3} was not most abundant(21%), G_{D1b} was the main ganglioside(34%) and G_{T1b} and G_{Q1b}(the spot between G_{T1b} and origin) made significant contributions. Non-parenchymal cells, in contrast to parenchymal cells, contained G_{M3} as the main component(72%) with the others

Table II Percentage distribution of ganglioside sialic acid in human liver, rat liver, parenchymal cells and non-parenchymal cells.

	Human liver(1)	Rat liver(3)	Parenchymal cells(3)	Non-parenchymal cells(3)
G_{M3}	80.1	61.1±3.3	21.2±3.9	71.9±5.3
G_{M2}	3.9	-	-	0.3±0.5
G_{M1}	0.8	3.7±3.9	1.7±0.4	6.4±0.1
G_{D3}	5.3	4.9±1.2	7.4±0.5	2.5±1.3
G_{D1a}	4.1	4.2±4.4	1.3±1.0	3.5±2.0
G_{D1b}	0.5	13.2±2.8	34.3±3.4	6.7±3.2
G_{T1b}	0.3	9.4±4.2	12.5±2.1	3.4±1.5
G_{Q1b}	-	2.2±3.9	19.3±4.3	3.4±1.6
Others	5.3	1.4±1.2	2.3±1.8	1.8±2.8

Values were obtained by densitometric scanning of TLC and represent mean±S.D. Figures in parentheses are numbers of samples.

all less than 10%. Thus the major ganglioside of the rat liver, G_{M3}, is derived mainly from non-parenchymal cells although the cells are in the minority in rat liver. Polysialo-gangliosides, G_{D1b}, G_{T1b}, G_{Q1b}, arise mainly from parenchymal cells. These results may be related to the fact that in mammalian liver, parenchymal cells originate from the endoderm and non-parenchymal cells from the mesoderm(25).

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