GANGLIOSIDES IN BLOOD SERUM OF NORMAL RATS AND MORRIS HEPATOMA 5123tc-BEARING RATS[1]

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Received October 20,1975

SUMMARY

The effects of malignancy upon blood serum ganglioside patterns were investigated. Lipids extracted from the blood serum of Morris hepatoma 5123tc-bearing rats were characterized by severalfold increases in the content of hematosides, monosialogangliosides and disialogangliosides, as compared with lipids extracted from the serum of normal rats. However, the content of trisialogangliosides in lipids extracted from the serum of cancer-bearing rats substantially decreased. In general, the change in the profile of gangliosides in blood serum reflects, but is less pronounced, than that observed in the comparison of Morris hepatoma tissue to normal liver tissue.

INTRODUCTION

The presence of gangliosides as normal constituents of blood plasma/serum in mammalia, including human beings, has been demonstrated by a number of investigators (1-5). It has been shown also that malignant tumors, spontaneous or transplanted, are characterized by the alteration of their ganglioside patterns, as compared with the normal tissues of their origin (6,7). For example, a number of different rat hepatomas have a higher content of low molecular weight gangliosides and a smaller content (or even complete lack) of high molecular weight gangliosides as compared with normal liver (8-12). In addition, the appearance (or increased amount) of the protein-lipid complex, tentatively called neoproteolipid-S (13,14), was observed in Morris hepatoma and other tumors. This complex consists of sialolipid, other lipids and

[1] This work was supported by NCI grants 5 RO 1 CA 15169 and 08748.

[2] We thank Dr. Kanematsu Sugiura, from our Cancer Center, for transplantation of the hepatomas, and Dr. David A. Wenger, University of Colorado Medical Center, for his generous gift of hematosides.

proteins (or polypeptides). The content of neoproteolipid-S in the blood serum of Morris hepatoma-bearing rats increases at least 5-fold as compared with normal controls. These observations, taken together, prompted us to investigate the possible effect of malignancy upon the blood serum gangliosides.

METHODS:

Lipid extraction and Folch partition of lipids. The experiments were conducted with blood serum obtained from male Morris hepatoma 5123tc-bearing rats with tumors transplanted 31-35 days earlier and serum from control male Buffalo rats. For each experiment, pooled samples of blood from several animals were analyzed. Sera were obtained by the conventional method and only those samples with no indication of hemolysis were analyzed for the content of gangliosides. Each serum sample was then extracted with 20 times its volume of chloroform:methanol (2:1, v/v at 4°). The extraction proceeded for 1 hr at 4° with occasional, vigorous shaking. After filtering, the solid residue was reextracted at room temperature with a solvent mixture 10 times the volume of the original serum sample. A chloroform:methanol mixture (1:1, v/v) with 3% water was used. The extraction proceeded for 1/2 hr, was filtered, and both extracts were combined; chloroform was then added to adjust the ratio of chloroform to methanol to 2:1 (v/v). The combined extract was cooled to 4-5° and washed with a volume of water 1/5 that of the total extract volume according to the method of Folch et al. (15). Only one washing was performed.

The upper phase of the Folch-wash was evaporated to dryness under reduced pressure (in a nitrogen atmosphere) in a rotary evaporator with a water bath at 45°. The residue was quantitatively transferred to a test tube by repeated washing of the flask with water and chloroform:methanol (1:1, v/v). The solution in the test tube was then evaporated to dryness in a water bath at 45° under a stream of nitrogen. The solid material, which contains most of the gangliosides extracted from blood serum, was suspended in a volume of water 1/300 to 1/500 that of the total upper phase Folch-wash volume. This was then dialyzed against water for 24 hr at 4-5° according to the procedure of Kanfer and Spielvogel (16). The contents were evaporated to dryness under a stream of nitrogen at 45° and redissolved in a measured volume of chloroform:methanol (1/1, v/v).

The lower phase of the Folch-wash was evaporated to a small volume under reduced pressure in a nitrogen atmosphere and quantitatively transferred into a volumetric flask. This fraction contains practically all "neutral" (simple) lipids, phospholipids and neutral glycosphingolipids; it also contains substantial amounts of gangliosides (hematosides and monosialogangliosides and/or its complexes). The concentration of the total lipids in this fraction was determined by weight. Measurements taken of the various ganglioside quantities, whether in the lower or upper phase of the Folch-wash, were standardized to the amounts that would be found in 100 mg of total lipid.

Determination of gangliosides in upper Folch phase. Purified ganglioside fractions obtained from the upper phase of the Folch-wash were subjected to analytical thin-layer chromatography (TLC). The gangliosides were applied to the chromatoplate in an amount equivalent to 20-40 mg of total lipid in the lower phase. Chromatoplates were prepared, 0.5 mm thick, from Silica Gel H (type 60, without binder, E. Merck, Darmstadt, Germany). The gangliosides on thin-layer chromatograms were identified by

their mobilities in relation to reference standards. Two different developing solvents were used: chloroform:methanol:0.25% aqueous $CaCl_2$, 60:35:8 (v/v) (17), and chloroform:methanol:2.5N NH_4OH, 60:45:9 (v/v) (18). Both TLC systems gave quantitatively similar results. Spots on the chromatograms were visualized with primuline spray under UV light (19) as adapted in our laboratory for glycosphingolipid detection (20). The ganglioside spots were scraped off, and the sialic acid content in them was determined with resorcinol reagent (21,22) without elution of the gangliosides from silica gel (23).

Determination of gangliosides in lower Folch phase. Quantitative determination of the gangliosides in the lower phase was performed in the following way. Twenty-forty mg of total lipid obtained from the lower phase were chromatographed on a small dry column. This column was packed with 0.5 g of dry silicic acid and then, on top, a 2 g mixture of dry silicic acid: Florisil (magnesium silicate), 1:1, (w/w) was layered. The inside diameter of the column was 0.6 cm. After packing, activation was at 175° for at least 2 hr. The chromatography column was eluted with 6 ml chloroform and 27 ml chloroform:methanol, 1:3, (v/v). The first 6 ml of eluate was discarded, and the remaining eluate (around 25 ml) was used for the determination of hematoside and monosialoganglioside amounts in this phase. This eluate was concentrated under a stream of nitrogen to a very small volume (200-300 µl) and applied to a silica gel chromatoplate. The chromatogram was developed with chloroform:methanol:0.25% aqueous $CaCl_2$, 60:35:8, (v/v) (17), and the separated gangliosides were quantitated as described above for the upper phase. Thus, the amounts of hematosides and monosialogangliosides in blood sera are the sum of their respective amounts in the upper and lower phases of the Folch-wash.

RESULTS AND DISCUSSION:

Table 1 shows the amount of different gangliosides in sera of normal rats and Morris hepatoma-bearing rats expressed in nanomoles per 100 mg of serum lipids. The content of monosialogangliosides and disialogangliosides increases around 4-fold in the serum lipids of tumor-bearing rats. An increase of hematosides is less pronounced, averaging around 2-fold. However, the amount of trisialogangliosides in the serum lipid of cancer-bearing rats decreases around 3-fold. Total amounts of lipid-bound sialic acid of serum lipids in cancer-bearing rats increase more than 2 times in spite of the decrease of the trisialogangliosides.

The total amount of lipids (per ml) in the serum of hepatoma-bearing rats is only slightly lower (by 6.7%) than in normal control rats. Therefore, the above described alteration of ganglioside patterns, expressed per 100 mg of serum lipids, is also valid when it is expressed per 100 ml of serum and is presented in this form in the last two columns of Table 1 for illustrative purposes.

TABLE I

Content of gangliosides in blood serum of normal rats and Morris hepatoma 5123tc-bearing rats

Results are expressed in nanomoles*

Gangliosides	Per 100 mg serum lipids			Per 100 ml serum**	
	Normal rats	Hepatoma rats	Hepatoma rats / Normal rats	Normal rats	Hepatoma rats
Hematosides	38 (36-40)	72 (61-85)	1.9	114	202
Monosialogangliosides	13 (9-17)	50 (34-59)	3.8	39	140
Disialogangliosides	19 (15-22)	76 (75-79)	4.0	57	213
Trisialogangliosides	12 (11-12)	4 (2-5)	0.3	36	11
Total lipid-bound sialic acid	124	287	2.3	372	804

*Results were obtained on 3-4 pooled serum samples. Each sample was analyzed 2-3 times. Results presented on the Table are the average of all determinations with range of values given in parentheses.

**Blood serum from normal rats contains on average 3 mg/ml lipids; serum from hepatoma-bearing rats contains on average 2.8 mg/ml lipids.

In general, the trend of alterations in the ganglioside profiles of blood sera from Morris hepatoma-bearing rats is a reflection of the alterations occurring in the tumor (11). The highest tumor elevations were seen in disialogangliosides and monosialogangliosides (34-fold and 15-fold, respectively) as compared with normal liver tissue. The hematoside concentration increases only moderately in Morris hepatoma (3-fold), whereas the trisialoganglioside level seems to decrease to unmeasurable levels in this tumor.

To what degree the alteration of ganglioside patterns in blood serum indicates the presence of all forms of malignancy in an organism is, at present, unknown. Most likely, the type of tumors which are characterized by the presence of neoproteolipid-S (13,14) may have substantial alterations of ganglioside biosynthesis and, as a result of that, one may also expect profound alterations of the gangliosides in the blood serum.

Recent publications (24-27) concerning the effect of malignancy upon sialic acid content in blood serum unfortunately provide data for total values of sialic acid released during analytic hydrolysis not only from gangliosides, but also from other sialic acid-containing compounds. Therefore, these data do not give information concerning the ganglioside levels and the effect of malignancy upon them so that conclusions drawn from these data are necessarily limited. Further studies on the effect of malignant tumors, including human tumors, upon blood plasma/serum ganglioside patterns are indicated since the observations presented in this communication may have practical implications as a potential diagnostic test for at least some forms of cancer.

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