Isolation and Characterisation of Glycolipids and Glycosaminoglycans from Meningiomas

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The glycolipids and glycosaminoglycans have been isolated and quantitatively estimated from meningeoma. The glycolipid concentrations in meningeoma are much lower compared to those from normal brain. The differential analysis of gangliosides showed higher concentration of GM₄ in meningeoma. Although the total amount of glycosaminoglycans of brain is comparable to that of meningeoma, there is a significant increase in heparan sulfate in the latter.

Introduction

Evidence for altered glycolipid composition of neoplastic tissues have been reported in the literature. Brante (1949) had reported increased amount of esterified cholesterol in intracranial tumours. Phospholipid changes in the brain tumours have been reported by several authors (Nayyar, McCaman and Heimburger, 1960; Lon-Cristensen, Clausen and Bierring, 1965; Wood and Harlow, 1970). Rapport et al. (1958, 1967) have isolated and showed the antigenic property of two ceramide dihexosides termed cytolipin H and R. Kosaki et al. (1958) have shown the presence of a new choline containing phospholipid from human neoplastic tissue which could not be demonstrated in normal tissue.

There are a very few reports on the quantitative data of gangliosides of brain tumours. Seifert and Uhlenbruck (1965) had reported altered ganglioside pattern in meningeoma. Lowden and Wolfe (1964) reported absence of gangliosides in glioblastoma multiforma. But Slagel, Dittmer and Wilson (1967) had shown a lower amount of gangliosides in human glial tumours.

The present report describes the quantitative data on the various glycolipids as well as glycosaminoglycan (GAG) content of the meningeomas. Meningiomas are the tumours of the central nervous system but are not of glial or neuronal origin. There is no report on the GAG content of the meningeomas in the literature. In this report, we have quantitatively estimated the total as well as the various GAG fractions from the meningeomas.

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Material and Methods

The tumour tissues were obtained in ice after surgical procedure. The tissues were washed with ice-cold normal saline and kept frozen at $-20^\circ$ until used. Sephadex G-25 and DEAE Sephadex were purchased from Pharmacia, Sweden. Hyaluronidase, hexosamines and chondroitin sulphates were obtained from Sigma Chemical Co., U.S.A. Chloroform, methanol, phenol and other solvents were distilled before use. Potassium chloride, sodium hydroxide, sodium chloride, cetyl pyridinium bromide and other chemicals were of analytical reagent grade. Gangliosides, cerebrosides, sulfatides and sphingomyelin were isolated from human brains of patients died from non-neurological disorders, were obtained at autopsy through the courtesy of Pathology department.

Extraction of glycolipids: The frozen tissue was extracted with 20 volumes of chloroform : methanol (2 : 1; v/v) overnight at room temperature. The residue was further extracted with 10 volumes of chloroform : methanol (1 : 2; v/v) for 6 h. at room temperature. The chloroform-methanol extracts were pooled and evaporated to dryness by flash evaporation at $38^\circ$. The residual lipid material was weighed and dissolved in 20 volumes of chloroform : methanol (2 : 1; v/v) and partitioned with 0.2 volumes of 0.74 per cent KCl solution. The upper aqueous phase was separated after short centrifugation. The lower phase was washed twice with 0.2 volume of “theoretical upper phase” containing no salt of Folch, Lees and Sloane-Stanley (1957). The aqueous phases were pooled and dialyzed at 4° for 24 h. against 4 changes of 1000 volumes of deionised distilled water. The material inside the dialysis bag was evaporated to dryness under reduced pressure at $38^\circ$.

The gangliosides in the aqueous phase was estimated by measuring N-acetyl-neuraminic acid (NANA) after hydrolysis at 80° in presence of 0.1 NH$_4$SO$_4$ for 60 min. according to the method of Warren (1959) as modified by Saifer and Gerstenfeld (1962). For differential analysis of gangliosides an aliquot of ganglioside solution containing 0.06—0.08 μ mole NANA was applied on 250 μ thick silica gel G plates and developed for 90 min. in a solvent system chloroform : methanol : 2.5 N NH$_4$OH :: 60 : 35 : 8 (v/v/v). After drying the plates at room temperature, they were exposed to iodine vapour. The iodine-positive spots were scraped quantitatively for NANA estimation as described above.

Sphingomyelin: The lower phase was dried under reduced pressure at $38^\circ$. 15-25 mg. of lower phase lipid was dissolved in 1 ml. of chloroform and mixed with 1 ml. of 0.5 N methanolic NaOH. After standing for 4 h. at room temperature, 1 ml. of 1.5 N methanolic HCl was added and the solution was mixed thoroughly and left for 30 min. To this acidified solution 3 ml. of chloroform and 1 ml. of distilled water was added and mixed thoroughly. Two phases were separated by short centrifugation and the upper aqueous phase was discarded. The lower phase was washed twice with 1 ml. portion of a mixture of methanol : water (1 : 1, v/v). The washed lower phase was evaporated to dryness under nitrogen. The dried residue was dissolved in a definite volume of chloroform. An aliquot (20-40 μl) of this solution was applied
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on a silica gel H plate of 250 μ thickness and developed with the solvent system chloroform : methanol : water 65:25:4 (v/v/v). After drying the plates in air, they were sprayed lightly with water to make the spots visible. The spot corresponding to the authentic sphingomyelin was marked. The dried spots were scraped quantitatively into test tubes and mixed with 0·8 ml., 70 per cent HC1O4 and heated at 187° for 3 h. The inorganic phosphorus thus liberated was measured according to the method of Bartlett (1959). μg. phosphorus × 25 = μg. sphingomyelin.

Cerebrosides and sulfatides: The cerebrosides and sulfatides were isolated and separated from mild alkali hydrolyzed lower phase lipid by a combination of florisel and DEAE-cellulose (acetate form) column chromatography according to the method of Rouzer, Kritchevsky and Yamamoto (1967). They were estimated by phenol-sulfuric acid method of Roughan and Batt (1968).

GAG were isolated from lipid-free dry tissue as described by Singh and Bachhawat (1968). GAG were precipitated by cetyl pyridinium bromide (CPB) and fractionated by differential extraction with increasing concentration of NaCl according to the method of Schiller, Slover and Dorfman (1961). Each CPB fraction was subjected to DEAE-Sephadex A-25 ion exchange chromatography as described by Schmidt (1962) using the same solvent systems reported by Singh and Bachhawat (1968). Uronic acid was measured by Dische’s (1947) carbazole method as modified by Bitter and Muir (1962). Hexosamines were estimated according to the method of Ludowieg and Benammann (1968). They were further identified by paper chromatography using a solvent system described by Mukherjee and Sri Ram (1964). Total sulfate was estimated by the method of Dodgson and Price (1962). Sulfamino group was estimated by nitrous acid-indole method of Lagunoff and Warren (1962). Gel filtration was carried out after hyaluronidase digestion on Sephadex G-25 to quantitate the hyaluronidase resistant material. Chondroitin-4-sulfate and chondroitin-6-sulfate were estimated according to the method of Mathew and Inouye (1961).

Results and Discussion

The glycolipid content of meningiomas is shown in Table I. As control we have used brain tissue from patients who died of non-neurological disorders. The grey and white matter of the brain were not separated. Meningiomas contained much lower amount of glycolipid compared to the brain, this is probably due to the non-neuronal origin of this type of tumours of the central nervous system.

The differential analysis of the various types of mono- , di- and trisialogangliosides are shown in Table II. The total ganglioside content of tumours is also very low compared to the brain. The most prominent feature is the higher amount of GM1, a monosialolactosylceramide ganglioside. In normal brain this ganglioside is present only in trace amount. But it has been shown by Brady and Mora (1970) that mouse cells lines transformed spontaneously tumourogenic or altered by infecting with SV 40 and polyoma viruses also showed that this type of alteration of the gangliosides. The ganglioside pattern is shown in Plate CXLI. The other glycolipids, such as
Fig. Ganglioside pattern. Chloroform : methanol : 2.5N Ammonia (60 : 35 : 8, v:v:v) was used as a solvent system. The spots were detected by Spraying Bial's Orcinol reagent, followed by heating at 120° for 15 min. C — Control, M — Meningioma.
cerebrosides, sulfatides and sphingomyelin are very low in concentration as compared to that of brain.

Table I. Concentration of various glycolipids.

<table>
<thead>
<tr>
<th>Glycolipids</th>
<th>Per g. of wet tissue</th>
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<tbody>
<tr>
<td></td>
<td>Control (brain)</td>
</tr>
<tr>
<td>Gangliosides</td>
<td>3·96 — 5·02</td>
</tr>
<tr>
<td>(µ mole sialic acid)</td>
<td>1·3 — 2·0</td>
</tr>
<tr>
<td>Sphingomyelin (mg.)</td>
<td>20·2 — 24·3</td>
</tr>
<tr>
<td>Cerebrosides (mg.)</td>
<td>5·0 — 6·4</td>
</tr>
</tbody>
</table>

Table II. Differential analysis of gangliosides.
(As percentage of total sialic acid)

<table>
<thead>
<tr>
<th>GM₃</th>
<th>GM₂</th>
<th>GM₁</th>
<th>GD₁₈</th>
<th>GD₁β</th>
<th>GT₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (brain)</td>
<td>0·9</td>
<td>3·2</td>
<td>20·6</td>
<td>42·6</td>
<td>16·7</td>
</tr>
<tr>
<td>Meningiomas</td>
<td>15·3</td>
<td>17·4</td>
<td>23·6</td>
<td>33·3</td>
<td>8·1</td>
</tr>
</tbody>
</table>

Nomenclature of gangliosides is according to Svennerholm, L.

The significance of the findings is difficult to correlate. In this report we have compared the glycolipids and GAG content of the brain with that of meningiomas. There is significant difference between the various glycolipid content of brain and the meningiomas. The difference could be tentatively taken as the regional or tissue difference between the brain as a whole and the meningioma. The higher concentration of GM₃ in meningiomas were also reported by Seifert and Uhlenbruck (1965) in their studies. These authors have not quantitatively estimated the GM₃ content either in the brain or in the meningioma but reported to have seen on thin-layer chromatographic separation of gangliosides.

This is the first report on the GAG content of the meningiomas. There is not much difference in the total GAG content between the control brain and the meningiomas. But there is significant difference among the different types of GAG content between the brain and the meningiomas (Table III). Of particular interest is the heparan sulfate content. It is 59 per cent in case of meningiomas as compared to 16 per cent in brain. There is also a large difference between the hyaluronidase resistant galactosamine containing GAG. This increase in heparan sulfate is of interest. It has been shown by Silbert (1963) that the predominating GAG in the mouse mast cell tumours is heparin and its low sulfated derivatives. It may be possible that increase in heparan sulfate content could be a marker in cases of meningioma. Heparan sulfate
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content of brain as well as of urine increases to 50 per cent in cases of genetic disorders like Sanfilippo syndrome (George and Bachhawat, 1970).

<table>
<thead>
<tr>
<th>GAG</th>
<th>Percentage of the total GAG</th>
<th>Control (brain)</th>
<th>Meningioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>37.4</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>15.8</td>
<td>59.4</td>
<td></td>
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<tr>
<td>Chondroitin sulfates</td>
<td>46.3</td>
<td>14.4</td>
<td></td>
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<tr>
<td>Hyaluronidase resistant galactosamine</td>
<td>0.5</td>
<td>12.2</td>
<td></td>
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<tr>
<td>containing</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total GAG (µg. uronic acid)</td>
<td>1435</td>
<td>1070</td>
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</table>

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References


