Fatty Acid Pattern of the Different Phosphoinositide Fractions in Human Meningiomas

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ABSTRACT

Previous studies showed no differences in the phospholipid content of human meningiomas compared to normal leptomeninges, but only a higher unsaturation degree in the individual phospholipid fractions of tumors.

Inasmuch as phosphoinositides play a role in the membrane responsiveness to numerous effectors, we studied the fatty acid pattern of the different phosphoinositide fractions of 14 human meningiomas of different histological origin.

The fatty acid analysis revealed remarkable differences among the histological types, and, above all, among the different phosphoinositide fractions of a single tumor class. The phosphoinositides derived from transitional meningiomas appeared to be the most saturated ones, because of their low arachidonic acid content. Furthermore, in all the meningiomas, long chain polyunsaturated fatty acids were present only in the phosphatidylinositol fractions and the polyphosphorylated compounds appeared to be significantly different from the corresponding monophosphorylated ones.

The possible significance of the different fatty acid distribution in the three phosphoinositide classes is discussed.

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Index Entries: Human meningiomas; phosphoinositides, in human meningiomas; fatty acid composition; polyunsaturated fatty acids; phosphoinositides, analysis of the different fractions.

INTRODUCTION

Meningiomas are among the more frequent tumors of CNS; in fact they account for about 15–20% of all primary intracranial tumors (Zülch, 1979). Substantial evidences suggest that meningiomas derive from meningeal arachnoid cells to which they are similar in many cytological features (Cervas-Navarro and Vasquez, 1969; Tani et al., 1972). Whereas the cytoskeletal and extracellular matrix components of the different histological classes of human meningiomas have been studied in detail (Halleday et al., 1985; Kartenbeck et al., 1984; Rutka et al., 1986; Yung et al., 1984), little is known about the plasma membrane components, in particular glycolipids and phospholipids, in spite of their important role in membrane architecture and cell-cell recognition, interaction, and growth.

The ganglioside content of human meningiomas, previously studied in our laboratories (Berra et al., 1983, 1990), showed no correlation between ganglioside distribution and histological classification, as reported also by Davidsson et al. (1989).

When we examined the phospholipid content and composition of 14 intracranial meningiomas of different histological origin, in comparison with normal leptomeninges (Riboni et al., 1984), we found that the total phospholipid content of tumors (expressed as organic phosphorus/mg DNA) was similar to that of the tissue from which they mainly derive. The distribution of the different phospholipid classes was different between normal leptomeninges and meningiomas; in tumors we found a decrease of sphingomyelin and an increase of phosphatidylcholine and of phosphoinositides (PtdIns). However, no correlation between phospholipid pattern and histological classification of tumors (performed according to Rubinstein, 1972) was found; only transitional meningiomas were different from the other classes, showing a lower phosphatidylserine content than leptomeninges.

The total and the individual phospholipid fractions isolated from meningiomas showed a higher content of unsaturated fatty acids; the only exception was the phosphoinositide fraction, where we observed an increase of palmitic acid and a decrease of oleic and mainly arachidonic acid (Riboni et al., 1984).

Meningiomas are considered to be not malignant tumors; but, although they do not metastasize, they may regenerate after surgical removal. This characteristic is most peculiar in some of the histological types of meningiomas. The possibility of a link between this regenerating ability, correlated to the histological classification, and the lipid composition of the plasma membrane is still unknown.

Inasmuch as phosphoinositide play a role in the membrane responsiveness to numerous effectors (for a recent review *see* Michell et al., 1989), the previously observed alterations in meningiomas (Riboni et al., 1984) might contribute to the impaired response to exogenous stimuli, a characteristic of tumors cells, and might determine different regenerating ability.

The phosphoinositide include 3 different compounds—phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PIP), and phosphatidylinositol-4,5-bisphosphate (PIP₂)—playing different physiological roles. In order to further characterize the difference among some of the different histological types of meningiomas, we fractionated the total phosphoinositide fraction derived from meningiomas of different histological classification, and analyzed the fatty acid profile in the single fractions.

MATERIALS AND METHODS

Materials

Silicic acid for column chromatography was from Mallinckrodt Inc. (Paris), high performance thin layer chromatography plates (HP-KF, 220 μ) were from Whatman (Clifton, NJ, USA). Reagents for gas chromatography were purchased by Carlo Erba (Milan), fatty acid methyl ester standards were from Nu Chek Prep. (Elysian, MN, USA), and phosphoinositide standard were from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Human Tissue Samples

Fourteen intracranial meningiomas obtained at the time of surgery (Unity of Neurosurgery-USL 10/D, Florence, Italy) from patients aged 40–60 yr were examined. Immediately after removal, the samples of tumors were frozen in liquid nitrogen, and stored at -80°C until analyzed. The histological classification of meningiomas was performed only after the biochemical analysis, so that we were unaware of the type of tumor we were analyzing. The meningiomas were then histologically classified according to Zülch (1979) and divided in : fibroblastic ($n = 7$), transitional ($n = 3$), and meningiotheliomatous ($n = 4$).

Methods

Total lipids were extracted from each tumor with the method of Bligh and Dyer (1959), with some modifications as a result of the use of acidified methanol as reported by Schacht (1981). Extraction from tissues was performed using 3 mL methanol:HCl (100:1)/chloroform (2:1 v/v) per g of tissue. To each mL of homogenate were then added 0.3 mL of 2.5N HCl and 0.3 mL chloroform. After mixing, the lower phase was

collected and the extract was applied to columns. The lipid extract was fractionated by silicic acid column chromatography according to Vance and Sweeley (1967) and phospholipids were totally eluted with methanol.

The whole phospholipid fraction was dried, redissolved in 100 μ L of methanol/chloroform/water (25:75:2 v/v) and applied on high performance thin layer chromatography plates previously impregnated with 1% potassium oxalate in methanol/water (2:3 v/v) and activated (15 min, 100°C). The plates were developed in chloroform/acetone/methanol/acetic acid/water (40:15:13:12:8 v/v) as described by Jolles et al. (1981). The spots corresponding to PI, PIP, and PIP₂ were visualized with iodine vapor and identified by comparison with cochromatographed standards. In both solvent systems, butylated hydroxytoluene 0.02% w/v was added to avoid lipid peroxidation. The scraped spots were extracted twice with 1.5 mL of methanol/chloroform/water (25:75:2 v/v) and dried under N₂.

The phosphoinositides were transmethylsterified with methanol/HCl (5% v/v) according to Stoffel et al. (1959). The resulting fatty acid methyl esters were gas chromatographed on a Varian mod. 3700 gas chromatograph equipped with a capillary column (30 m $\times$ 0.25 mm id), filled with a thermostable stationary phase (SP 2340 0.2 μ m) and heated at a programmed temperature (160–210°C) with a gradient of 8°C/min. The carrier gas (helium) flow rate was 2 mL/min. The gas chromatographic peaks were identified on the basis of their retention time ratio relative to methyl stearate and predetermined on authentic samples. Gas chromatographic traces and quantitative evaluations were obtained using a Spectra Physics (San Jose, CA) mod. 4100 computing integrator.

Data are expressed as means $\pm$ SD; owing to the low number of samples, data of transitional meningiomas are presented as a range. Statistical differences were evaluated using Student's *t*-test, only between fibroblastic and meningiotheliomatous meningiomas.

RESULTS

Data on the fatty acid composition of the PI, PIP, PIP₂ fractions of the different histological types of human meningiomas are reported in Tables 1, 2, and 3 respectively.

The most remarkable difference between PI and PIP or PIP₂ is the presence of long chain polyunsaturated fatty acids (PUFA) in the PI fraction only. This difference is particularly evident in the fibroblastic meningiomas. In the PI fraction, the arachidonic acid percent content is higher in the meningiotheliomatous meningiomas than in the fibroblastic type (Fig. 1); in this fraction, the decrease in linoleic acid content and the increase in palmitic acid amount in fibroblastic meningiomas with respect to meningiotheliomatous ones are balanced by a significant increase in oleic acid content (Table 1). As a consequence, the DBI value is similar

Table 1
PI Fatty Acid Composition (% w/w) of Different Types of Human Meningiomas

Fatty Acid	Fibroblastic (<i>n</i> = 7)	Meningotheliomatous (<i>n</i> = 4)	Transitional (<i>n</i> = 2)
16:0	24.5 ± 2.5	17.9 ± 3.8**	27.6 – 37.4
16:1	1.2 ± 1.2	1.7 ± 1.0	4.1 – 1.1
18:0	18.0 ± 2.7	20.9 ± 0.5	26.1 – 32.8
18:1	31.2 ± 4.9	24.9 ± 3.6*	16.4 – 17.1
18:2	2.9 ± 0.7	10.6 ± 2.6***	3.6 – 4.6
20:4	19.2 ± 1.6	21.7 ± 1.7*	14.5 – 14.7
22:4n-6	1.4 ± 1.3	0.8 ± 1.0	tr
22:5n-6	0.3 ± 0.2	tr	tr
22:5n-3	0.3 ± 0.2	tr	tr
22:6n-3	1.3 ± 2.1	1.3 ± 0.7	tr

PI fatty acid composition analysis was performed in the conditions reported in Materials and Methods. Values are means ± SD except for transitional meningiomas where only the range is reported. The number of samples examined is indicated within the brackets. Tr means a negligible amount of the corresponding fatty acid (<0.1%). Statistical differences between fibroblastic and meningotheliomatous meningiomas were evaluated using Student's *t*-test: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Table 2
PIP Fatty Acid Composition (% w/w) of Different Types of Human Meningiomas

Fatty Acid	Fibroblastic (<i>n</i> = 7)	Meningotheliomatous (<i>n</i> = 4)	Transitional (<i>n</i> = 3)
16:0	33.7 ± 2.2	29.9 ± 8.4	19.2 – 22.5
16:1	7.2 ± 3.2	8.0 ± 3.1	1.5 – 3.2
18:0	22.6 ± 2.3	23.1 ± 1.6	28.6 – 32.3
18:1	20.9 ± 1.1	27.1 ± 7.0*	26.8 – 30.5
18:2	9.9 ± 2.9	6.1 ± 0.9	7.2 – 11.9
20:4	5.5 ± 1.8	5.8 ± 1.9	7.8 – 9.6

PIP fatty acid analysis was performed in the conditions reported in Materials and Methods. Values are means ± SD, except for transitional meningiomas where only the range is reported. The number of samples examined is shown within the brackets. Statistical differences between fibroblastic and meningotheliomatous meningiomas were evaluated using Student's *t*-test: **p* < 0.05.

between the two types of meningiomas (Fig. 2). The DBI values for each group were calculated from data in Tables 1, 2, and 3, taking the concentration of each fatty acid, multiplying it by the number of double bonds in the fatty acids, and adding up all the scores for all the fatty acids.

In the PIP fraction, the differences in fatty acid composition are less evident than in the PI and PIP₂ fractions (Table 2); in the fibroblastic and meningotheliomatous meningiomas, the saturation increases in comparison to the PI fraction (Fig. 2).

The fatty acid patterns of PIP and above all of PIP₂ fraction markedly

Table 3
PIP₂ Fatty Acid Composition (% w/w) of Different Types of Human Meningiomas

Fatty Acid	Fibroblastic (n = 7)	Meningotheliomatous (n = 4)	Transitional (n = 2)
16:0	29.0 ± 0.9	22.7 ± 2.5***	34.7 – 35.6
16:1	6.3 ± 1.0	3.5 ± 1.2**	4.9 – 5.3
18:0	24.3 ± 1.6	22.8 ± 1.6	23.2 – 23.6
18:1	25.3 ± 1.6	22.3 ± 1.6*	24.3 – 24.4
18:2	6.6 ± 0.4	24.4 ± 2.8***	4.1 – 7.4
20:4	8.4 ± 1.4	4.2 ± 1.1***	3.7 – 3.7

PIP₂ fatty acid analysis was performed in the conditions reported in Materials and Methods. Values are means ± SD except for transitional meningiomas where the range is reported. The number of samples examined is shown within the brackets. Statistical differences between fibroblastic and meningotheliomatous meningiomas were evaluated using Student's *t*-test: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

differ in the proportional content of arachidonic acid in comparison to PI fraction in all the histological types of meningiomas (Fig. 1). In fact, a dramatic decrease in arachidonic acid content is shown in comparison to the PI fraction.

In the PIP₂ fraction of meningotheliomatous meningiomas, the higher DBI value, observed notwithstanding the decrease in arachidonic acid content, is owing to the unexpected increase in linoleic acid content, which is about four times higher than in the PIP₂ fractions of fibroblastic meningiomas.

DISCUSSION

The analysis of the fatty acid compositions revealed significant differences among the different types of human meningiomas in all the phosphoinositide classes. Although it is difficult to attribute a functional significance to each modification, this different acidic pattern must be taken into account, in fact, the different histological origin of the tumors seems to influence an intrinsic property of the membrane, such as the lipid composition.

The acidic pattern of PIP₂ is of particular interest. The normal configuration of PIP₂ is reported to be 1-stearoyl, 2-arachidonoyl, at least in liver (Holub and Celi, 1984). The fatty acid analysis of the PIP₂ fraction of human meningiomas revealed the presence of interesting differences in comparison to data reported in the literature for the normal tissues (Holub and Celi, 1984). In fact, in our study, the PIP₂ 2-position might be prevalently occupied by oleic acid in the fibroblastic type and by oleic and/or linoleic in the meningotheliomatous one. The high content of palmitic acid might indicate that also the 1-position could be differently esterified than in normal tissues. Similiar conclusions could be drawn

Fig. 1. Arachidonic acid % content in the phosphoinositide fractions of the different types of human meningiomas. Data were calculated by gas chromatographic analysis as reported in Tables 1, 2, and 3 and are expressed as means $\pm$ SD except for transitional meningiomas where, owing to the low number of samples, only the mean value is given. The number of samples examined is reported within the brackets. Statistical evaluations were performed between fibroblastic and meningotheliomatous meningiomas using Student's *t*-test: °*p* < 0.05, **p* < 0.001.

also for the PIP₂ fraction of transitional meningiomas, whose oleic acid relative amount is similar to the amount reported in the fibroblastic type.

Inasmuch as we were unaware of the histological type of the tumors examined until the end of the experimental analysis, we discovered later on that only three samples were classified as transitional meningiomas. Therefore, owing to the very low number of samples of transitional meningiomas examined, it is impossible to perform a statistical analysis for this type of tumors.

We did not find any polyunsaturated fatty acid in the PIP and PIP₂ fractions derived from all the histological types of meningiomas; these findings are in agreement with the results of Baker and Thompson (1972) who reported the absence of polyunsaturated fatty acids longer than arachidonic acid in the PIP and PIP₂ fractions derived from rat brain phospholipids. Moreover, Haycock and Evers (1988) found that polyphosphoinositides from normal rat brain had a significantly lower mol% of arachidonate than did PI.

The particular fatty acid pattern of PIP₂ and the differences among PI, PIP, and PIP₂ fatty acid composition, in all the histological types of meningiomas, deserve some other comments and considerations.

Fig. 2. Double bond index (DBI) in the phosphoinositide fractions of the different types of human meningiomas. The DBI for each group was calculated by taking the concentrations of each fatty acid obtained by gas chromatographic analysis, multiplying it by the number of double bonds in the fatty acid, and adding up all the scores for all the fatty acids. Data are means $\pm$ SD, except for transitional meningiomas where, owing to the low number of samples, only the mean value is reported. The number of samples examined is reported within the brackets. Statistical evaluations were performed between fibroblastic and meningotheliomatous meningiomas using Student's *t*-test: **p* < 0.001.

Receptor-stimulated hydrolysis of phosphoinositide is coupled to phosphoinositide resynthesis from phosphatidic acid (PtdOH) and inositol. Since PtdOH is a precursor for all glycerolipids, this process does not readily account for the efficient recycling into phosphoinositide of the PtdOH arising from agonist-stimulated phosphoinositide breakdown (Fain and Berridge, 1979). This phenomenon seems now to be accounted for by the occurrence of a metabolically segregated small pool of PIP₂. These observations might be correlated to our data that suggest, on the basis of the fatty acid distribution, the possible existence of different pools of PI, PIP, and PIP₂, the last two not directly derived by a simple PI phosphorylation.

Furthermore, although we did not examine the diacylglycerol (DAG) fatty acid composition, our data on PIP₂ acyl composition could suggest the possible existence of a DAG family whose configuration is not always or only the typical one. This uncommon DAG configuration could lead to a different protein kinase C (PKC) stimulation and to a reduced arachidonic acid release from DAG.

Recent evidence indicates that the pathway involving phosphoinositide breakdown could function as a key signaling route that controls cell proliferation, both normal and malignant, and therefore that this pathway might be the site of action of many oncogenes (Jaken, 1989).

The possibility that cell transformation might be associated with aberrant regulation of PKC activity has been given attention. An increase in steady state DAG levels has been associated with both *ras*- and *sis*-transformed fibroblasts (Wolfman and Macara, 1987). An increase in particulate vs soluble PKC ratio was reported for growing and transformed fibroblast cell lines with respect to confluent cultures. This increase in membrane-associated PKC was suggested to be linked to increased DAG levels of the transformed and growing fibroblasts (Halsey et al., 1987).

In meningiomas the regulation of the phosphoinositide cycle might therefore constitutively produce a modified 1,2-DAG, which could be slowly removed by DAG kinase, as reported by MacDonald et al. (1988), leading to a permanent activation of PKC; in other words, the cells would be generating their own tumor promoter.

In conclusion, we can hypothesize in human meningiomas: (a) a modification of eicosanoid metabolism, due to the DAG fatty acid profile which will be different from the normal one; (b) a possible different affinity of the phosphoinositide-specific phospholipase C (phosphoinositidase C) for the fatty acid modified PIP₂; and (c) a slower phosphorylation of the modified 1,2-DAG to PtdOH, as demonstrated by MacDonald et al. (1988); in this case, PKC will be maintained for a longer period in the active state.

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