ORIGINAL ARTICLE

Can Behenic Acid (C22:0) Levels be a Prognostic Factor in Glial Tumors?

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ABSTRACT: ***Background:*** Inhibition of fatty acid synthase leads to apoptosis in cancers, which leads to high levels of fatty acid synthesis. This indicates that cancer cells depend on fatty acid in order to survive. In this study, we investigated whether or not there was a relationship between the glial tumor grade and free fatty acid level of tumor tissue. ***Methods:*** Twenty patients who had high grade glial tumors and 20 patients who had low grade glial tumors, were included in the study. Tumors samples were obtained intraoperatively in order to measure the fatty acid levels. The fatty acids were studied in three groups: saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. They were analyzed with gas chromatography. ***Results:*** The oleic acid, linoleic acid, eicosadienoic acid, arachidonic acid, and docosadienoic acid levels were high in the tumor tissue of low grade tumors. The myristic acid, palmitic acid, stearic acid, alpha linoleic acid, eicosenoic acid, dihomo-gamma-linolenic acid, docosahexaenoic acid, and ceramide levels were high in the tumor tissue of high grade glial tumors. However, none of these high values were statistically significant. The high values of behenic acid, a saturated fatty acid, in low grade glial tumors were statistically significant. ***Conclusion:*** High levels of behenic acid in patients with low grade glial tumor is important as it indicates persistence of the tissue integrity and tissue resistance. Behenic acid levels can be a prognostic factor in glial tumors.

RÉSUMÉ: Le taux d'acide béhénique (C22:0) peut-il constituer un facteur de pronostic dans les tumeurs gliales ? ***Contexte :*** L'inhibition de l'acides gras synthase provoque l'apoptose dans les cancers ce qui entraîne un taux élevé de synthèse des acides gras. Ceci indique que les cellules cancéreuses dépendent des acides gras pour leur survie. Dans cette étude, nous avons examiné s'il existait une relation entre le grade de la tumeur gliale et le niveau d'acides gras libres dans le tissu tumoral. ***Méthode :*** Vingt patients porteurs de tumeurs gliales de haut grade de malignité et 20 patients porteurs de tumeurs gliales de bas grade de malignité ont été inclus dans l'étude. Des échantillons de tumeurs ont été obtenus pendant la chirurgie afin de mesurer les niveaux d'acides gras. Les acides gras ont été étudiés en trois groupes : les acides gras saturés, les acides gras monoinsaturés et les acides gras polyinsaturés. L'analyse a été réalisée par chromatographie en phase gazeuse. ***Résultats :*** les niveaux d'acide oléique, d'acide linoléique, d'acide eicosadiénoïque, d'acide arachidonique et d'acide docosadiénoïque étaient élevés dans le tissu tumoral provenant des tumeurs de bas grade de malignité. Les niveaux d'acide myristique, d'acide palmitique, d'acide stéarique, d'acide alpha-linoléique, d'acide eicosanoïque, d'acide dihomo-gamma-linolénique, d'acide docosahexaénoïque et de céramides étaient élevés dans le tissu tumoral provenant de gliomes de haut grade de malignité. Cependant, aucune de ces valeurs élevées n'atteignait le seuil de la signification au point de vue statistique. Les valeurs élevées d'acide béhénique, un acide gras saturé, dans les tumeurs gliales de bas grade de malignité étaient significatives au point de vue statistique. ***Conclusion :*** Des niveaux élevés d'acide béhénique chez les patients atteints de tumeurs gliales de bas grade de malignité sont importants parce qu'ils indiquent la persistance de l'intégrité du tissu et sa résistance. Les niveaux d'acide béhénique peuvent constituer un facteur de pronostic chez les patients atteints de tumeurs gliales.

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The role of fatty acids in tumor formation differs according to their structures. These differences have been only partially understood. For example, fatty acids have various effects on the synthesis and metabolism of prostaglandins (PGs). Eicosapentaenoic acid (EPA) has well-known anti-inflammatory effects that arise through the impairment of cytokine synthesis and release.[1] Polyunsaturated fatty acids (PUFAs) play a role similar to an intracellular second messenger in cell death, proliferation, and signaling in normal and altered cells. PUFAs stimulate tumor regression and apoptosis in human gliomas.[2] Gamma-linolenic acid (GLA) inhibits the increase in vascularization, which enables the nutrition and growth of the tumor. The increased fatty acid synthase (FAS) activity and the different roles of fatty acids in cancer tissue make the studies on this topic more interesting. Fatty acid synthase is defined as a prognostic factor in cancer, and the FAS level indicates endogenous fatty acid synthesis.[3] The inhibition of FAS leads to apoptosis in cancer, which leads to high levels of fatty acid synthesis. This indicates that cancer cells depend on fatty acid in order to survive.[4-6]

In this study, we investigated whether or not a relationship exists between the glial tumor grade and the free fatty acid levels of tumor tissue.

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Material and Method

Twenty patients who had high grade astrocytic tumors (anaplastic - glioblastom multiforme) and 20 patients who had low grade astrocytic tumors (grade II), who were operated on in our clinic were included in the study. Of the 40 patients participating in the study, 24 (60%) were male and 16 (40%) were female, aged between 13-74 years.

Approximately, 2 grams of tumor sample were obtained intraoperatively from each patient in order to measure the fatty acid levels in the tumor tissue.

The fatty acids were studied in three groups: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 1).

Lipid extraction and preparation of fatty acid methyl esters

Lipid extraction from tissue samples was performed with the Hara and Radin method, which uses a 3:2 volume/volume (v/v) hexan isopropranonol mixture.[7] The fatty acids in lipids were converted to methyl ester derivatives, which are not polar and have a volatile and stable structure, in order to perform a gas chromatographic analysis.[8]

To prepare the methyl ester, the lipid extract in the hexan/isopropranolol phase was transferred into 30 ml test tubes, which did not percolate. Five ml of 2% methanolic sulphuric acid was added and mixed well with a vortex. This mixture was left for methylation in the incubator at 50ºC for 15 hours. The tubes were removed from the incubator, cooled to room temperature, and mixed well by adding 5 ml of 5% sodium chloride. Fatty acid methyl esters were extracted with 5 ml hexan, and the hexan phase was placed into the pipette, treated with 2% potassium bicarbonate ($KHCO_3$) and kept for four hours to allow the phases to separate. The solution of the mixture that contained methyl esters was then subjected to evaporation at 45ºC and under nitrogen flow, dissolved with 1 ml of hexan, placed into 2 ml autosampler covered vials, and analyzed with gas chromatography.

Gas chromatographic analysis of fatty acid methyl esters

After the fatty acids in the lipid extract were converted to methyl esters, they were analyzed with Shimadzu GC 17 Ver. 3 gas chromatography. For this analysis, a Machery-Nagel capillary column measuring 25 µ, with a 0.25 µm inner diameter and Permabond 25 micron film thickness was used. During the analysis, the temperature of the column was kept at 120–220°C, the injection temperature was kept at 240°C, and the detector temperature was kept at 280°C. Nitrogen was used as the carrier gas. The mixture belonging to the standard fatty acid methyl esters was injected before the analysis of the fatty acid methyl esters of the samples, and the retention time of each fatty acid was determined. After this procedure, an analysis of the fatty acid methyl esters of the samples was performed.

Table 1: The fatty acids groups

SFA: Saturated fatty acids	**MUFA: monounsaturated fatty acids**	**PUFA: Polyunsaturated fatty acids**
C14:0: Myristic acid	C16:1ω7 (Palmitoleic acid)	C18:3ω3 (α-linolenic acid)
C16:0:Palmitic acid	C18:1ω9 (Oleic acid)	C20:3 (Eicosatrienoic acid)
C18:0:Stearic acid	C20:1ω9 (Eicosenoic acid)	C22:6ω3 Docosahexaenoic acid (DHA)
C22:0:Behenic acid	C24:1ω9 (Nervonik acid)	C18:2ω6 (Linoleic acid)
		C20:2ω6 (Eicosadienoic acid)
		C20:4ω6 (Arachidonic acid)
		C22:2ω6 (Docosadienoic acid)

Table 2: Fatty acid level values in tumor tissue of patients with glial tumors

Fatty Acids	TYPE		
	Low $\overline{X} \pm S_{\bar{x}}$ **(n=23)**	High $\overline{X} \pm S_{\bar{x}}$ **(n=17)**	p<0.05
C14:0	1.012 ± 0.126	1.235 ± 0.145	-
C16:0	23.652 ± 0.700	24.881 ± 0.815	-
C16:1	4.477 ± 0.191	4.581 ± 0.222	-
C18:0	15.758 ± 0.750	16.346 ± 0.872	-
C18:1	24.148 ± 0.574	22.933 ± 0.667	-
C18:2	5.484 ± 0.588	4.546 ± 0.684	-
C18:3	0.526 ± 0.074	0.529 ± 0.087	-
C20:1	0.983 ± 0.095	1.081 ± 0.110	-
C20:2	1.131 ± 0.081	1.129 ± 0.094	-
C20:3	2.079 ± 0.193	2.099 ± 0.225	-
C20:4	12.047 ± 0.592	11.830 ± 0.688	-
C22:0	0.521 ± 0.026	0.378 ± 0.030	*
C22:2	3.761 ± 0.367	3.021 ± 0.426	-
C22:6	4.455 ± 0.352	4.938 ± 0.410	-
C24:1	0.715 ± 0.128	0.767 ± 0.149	-
SFA	41.362 ± 0.782	43.268 ± 0.909	-
MUFA	30.539 ± 0.669	29.963 ± 0.778	-
PUFA	28.411 ± 1.005	27.900 ± 1.169	-

* p<0.05

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The results were determined as the percentage amount for each fatty acid among the total fatty acids. Calculations were made using the GC solution 2.3 program.

Statistical method

The SAS 9.2 package program was used for statistical analysis of data obtained from the study. The variance analysis was performed in order to determine the causes of the difference of the mean values of the groups from the general mean. The Duncan's multi-comparison test was used to determine the differences between the groups of more than two (SAS, 2010.SAS/STAT Software: Hangen and Enhanced, Version 9.2, SAS, Inst. Inc. Cary, N.C. USA).

Results

The oleic acid (C18:1), linoleic acid (C18:2), eicosadienoic acid (C20:2), arachidonic acid (C20:4), and docosadienoic acid (C22:2) levels were high in the tumor tissue of low grade tumors. The myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), alpha linoleic acid (C18:3), eicosenoic acid (C20:1), dihomo-gamma-linolenic acid (C20:3), docosahexaenoic acid (C22:6), and ceramide (C24:1) levels were high in the tumor tissue of high grade glial tumors. However, none of these high values were statistically significant (Table 2).

The high values of C22:0 (behenic acid (BA)), a saturated fatty acid, in low grade glial tumors were statistically significant (Table 2).

In the overall comparison of MUFA, PUFA, and SFA, there was no statistically significant relationship between the glial tumor grade and the fatty acid groups.

Discussion

There are some mechanisms and opinions about the role of fatty acids in carcinogenesis. They have been reported to be effective through changing the membrane viscosity, due to their toxic effects or metabolites, due to the high caloric effect, through lipid peroxidation, and with gene regulation.[9-13]

Biological membranes are composed of double lipid layers, which contain fatty acid chains in the core. This hydrophobic structure works as a barrier and prevents the irregular movements of ions and limits the random passage of metabolic products through the membrane. Proteins embedded in the membrane (enzymes, receptors, and carrier proteins) also interact with fatty acids.[14] While more than one type of fatty acid chain constitute the lipid layer, the majority of fatty acids present in this structure consist of 16 and 22 carbon atoms.[15] While a normal membrane structure contains saturated fatty acids at a rate of 35-40%, the remainder is composed of unsaturated fatty acids and contains double bonds between 1 and 6. The barrier function of the membrane changes when the rate of saturated and unsaturated fatty acid chains changes and significant changes occur in the signaling response of the proteins embedded in the membrane.[9,14-16] C22:0 is a saturated fatty acid of the SFA group, which is essential for the integrity of the cell membrane. Cells show resistance to cancer with membrane viscosity. Membrane viscosity has important roles in inter-cellular transmission, cell proliferation, and cell-to-cell adhesion. PUFAs and SFAs provide membrane viscosity.[6,16-18] In our study, the C22:0 level was found to be high in tumor tissue. This high level was also statistically significant. We consider high levels of C22:0 in patients with low grade glial tumors as important, as it indicates persistence of the tissue integrity and tissue resistance. Can C22:0 fatty acid levels be a prognostic factor in glial tumors? This hypothesis must be supported with further studies.

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