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Sphinganine in Sphingomyelins of Tumors and Mouse Regenerating Liver

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Abstract—Contents of sphingenine (sphingosine) and sphinganine were studied in sphingomyelins of transplantable mouse tumors (hepatoma-22, melanoma B16, Lewis lung carcinoma, intestine carcinoma) and rat nephroma RA. The content of sphinganine was increased in sphingomyelins of hepatoma-22 and nephroma RA compared to sphingomyelins of liver and kidneys. Significant contents of sphinganine were also found in sphingomyelins of other studied tumors. The content of sphinganine in regenerating mouse liver (30 h after hepatectomy) was normal. The data suggest that disorders should exist in biosynthesis of sphingoid bases in tumors but not in normal rapidly proliferating tissue.

Key words: sphinganine, sphingosine, sphingomyelin, tumor, regenerating liver

Sphingolipids are indispensable components of all eucaryotes. The term "sphingolipids" includes hundreds of compounds with various structures but with a sphingoid base in common. The main sphingoid in human and animal sphingolipids is D-*erythro*-sphingenine (sphingosine) with an 18-carbon chain and a *trans*-double bond in the position 4 — (2S,3R,4E)-2-amino-4-octadecene-1,3-diol. Its precursor in biosynthesis is sphinganine, which has no double bond. The sphinganine content in sphingolipids of normal tissues is very low (1-5%). However, we have found considerable amounts of sphinganine in ceramides of certain tumors (various human ovary carcinomas, the transplantable mouse hepatoma-22) [1-3]. Because dihydroceramides (sphinganine-containing ceramides), unlike ceramides, fail to inhibit proliferation and stimulate differentiation and cell apoptosis (see [4] and the literature cited in it), i.e., are not tumor suppressors [5, 6], it was suggested that the pool of "tumor" ceramides should be different in its ability to regulate cell growth. In fact, the antiproliferative activity of the ceramide pool isolated from human ovary carcinoma was lower than that of ceramides from the homologous normal tissue [7].

It was interesting to elucidate whether an increased amount of sphinganine in sphingolipids was specific only for tumor growth or for rapid proliferation of normal cells as well. Thus, the present work was designed to study the composition of sphingoid bases in sphingomyelins of transplantable animal tumors (rat nephroma RA, hepatoma-22, melanoma B16, Lewis lung carcinoma, large intestine carcinoma of mice) and of regenerating mouse liver.

MATERIALS AND METHODS

Mice weighing 18-20 g were transplanted subcutaneously with the following tumors: the solid hepatoma-22 (C3H mice), melanoma B16 (C_{57}Bl/6 mice), Lewis lung carcinoma (C_{57}Bl/6 mice), large intestine carcinoma (Balb/c mice). Nephroma RA was transplanted to Wistar rats weighing 120-130 g. All of the tumors were isolated on the 15-16th day after transplantation. Partial hepatectomy was carried out in C3H mice as described in [8], and the regenerating liver was isolated 30 h after the operation.

Lipids were isolated from the tissues by repeated extractions with $CHCl_3$–CH_3OH mixtures (2 : 1 and 1 : 2 v/v) until they were completely extracted. The lipid extract of each tissue was washed with water as described in [9]. After evaporation of the organic layer, the lipids were methanolyzed for 24 h with 0.2 M NaOH in CH_3OH at 20°C to degrade glycerolipids. After methanolysis, the reaction mixture was neutralized with 0.35 M AcOH in CH_3OH and evaporated. Sphingomyelin was isolated from the mixture by three-step thin-layer chromatography on

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silica gel as described earlier [2]. Sphingoid bases isolated from sphingomyelins of each tissue were analyzed with a Chrom-5 chromatograph (Czechia) on a column with 3% OV-1 on W-HP chromosorb (80-100 mesh) at 230°C.

Phospholipids and ceramides were quantitatively determined as described in [3]. Protein was determined by the method of Lowry et al. [10].

RESULTS AND DISCUSSION

Tumors are known to contain an increased amount of sphingomyelin compared to the homologous normal tissues (see review [11] and the literature cited in it). We showed earlier that, although the content of ceramide (which is a biosynthetic precursor of sphingomyelin and also a product of its metabolism) was changed in tumors, these changes were not regular. Thus, the content of ceramides was significantly decreased in human ovary tumors [1, 2] and increased in mouse hepatoma-22 [3] and in rat hepatoma RA [12] compared to normal tissues.

Also, no regular changes in the contents of sphingomyelins and ceramides were found in the tumors studied in the present work: the molar ratio sphingomyelin/ceramide for the tumors varied in the range 1.1-6.4, whereas for normal tissues (mouse liver, rat kidneys) this ratio was 2.5-5 (calculated per mg protein).

However, the studied tumors were characterized by an increased amount of sphinganine in sphingomyelin, similarly to the earlier finding for ceramides [1-3]. Because in addition to sphingosine and sphinganine, in nephroma RA, large intestine carcinoma, and in kidneys a certain amount of phytosphingosine and sphingoids with chain of another length was also found, the molar ratio sphingenine/sphinganine is given in Table 1. The data presented show that the content of sphinganine (relatively to sphingosine) is increased in sphingomyelins from mouse hepatoma-22 and rat nephroma RA compared to the corresponding normal tissues, and this results in a shift in the molar ratio sphingenine/sphinganine towards sphinganine. It was impossible to compare other studied tumors to the homologous normal tissues. However, the data presented also show that the content of sphinganine is significant in sphingomyelins from melanoma B16, Lewis lung carcinoma, and large intestine carcinoma of mice, and in the latter case the amount of sphinganine was a little higher than the amount of sphingenine. We found earlier a significant amount of sphinganine (more than 10%) in sphingomyelins from Woker carcinosarcoma, hepatoma-27, and Jensen rat sarcoma [13]. It was suggested that sphinganine should be inherent to any rapidly proliferating tissue, and, consequently, it should be also present in sphingomyelin from mouse regenerating liver during active mitosis (30 h after hepatectomy). Changes in the sphingomyelin content in the liver regenerate (Table 1) suggest changes in the sphingomyelin biosynthesis during the proliferation. However, data of gas-liquid chromatography of sphingoid bases of sphingomyelin from the mouse regenerating liver indicated that the sphinganine content was virtually unchanged from that in sphingomyelin from normal liver but was significantly lower than in sphingomyelin from hepatoma-22 (Table 2). This indicates that, although sphingomyelin biosynthesis is increased at the rapid proliferation of hepatocytes during active mitosis, the structure of its sphingoid bases is not changed compared to

Table 1. Contents of sphingomyelin and its sphingoid bases in normal and regenerating mouse liver, in transplantable mouse tumors, and in rat kidneys and nephroma

Tissue	Sphingomyelin*, % of total P	Molar ratio sphingenine/sphinganine in sphingomyelin
Liver (normal) (9)	3.8 ± 0.1	24 : 1
Liver regenerate (15)	8.2 ± 0.5	24 : 1
Hepatoma-22 (15)	7.0 ± 0.1	3 : 1
Melanoma B16 (15)	12.9 ± 0.8	7 : 1
Lewis lung carcinoma (18)	14.4 ± 0.6	10 : 1
Large intestine carcinoma (15)	17.5 ± 0.6	0.9 : 1
Rat kidney (9)	10.4 ± 0.1	65 : 1
Nephroma RA (9)	12.2 ± 0.4	5 : 1

Note: Here and in Table 2 the number of animals is shown in parentheses; for the analysis the total pool of sphingoids isolated from sphingomyelins of each tissue was used.

* Mean values for five determinations ± standard deviation are presented.

Table 2. Contents of sphingenine (sphingosine) and sphinganine in sphingomyelins from normal and regenerating liver and from mouse hepatoma-22

Tissue	Sphingenine, %	Sphinganine, %
Liver (normal) (9)	96	4
Liver (regenerate) (15)	96	4
Hepatoma-22 (15)	76	24

normal, i.e., the activities of enzyme systems involved in the biosynthesis of sphingoid bases are not changed compared to normal. It seems that the tumor growth (hepatoma-22, etc.) is associated with functional disorders of these enzyme systems, in particular, the activity of a recently found dihydroceramide desaturase [14-16] responsible for insertion of the *trans*-double bond into position 4,5 of the hydrocarbon chain of a sphingoid base dihydroceramide seems to be decreased, and this results in accumulation of sphinganine in sphingolipids.

Thus, the findings of the present work indicate that the presence of a significant amount of sphinganine, which is a saturated analog of sphingosine, is specific only for sphingolipids (in particular, for sphingomyelin) from tumors but not from rapidly proliferating tissues (at least during active mitosis). However, the possibility must not be ruled out that the sphinganine content in sphingolipids (relative to sphingosine) can be increased in other diseases. To elucidate this, further studies are required.

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REFERENCES

1. Dyatlovitskaya, E. V., Andreasyan, G. O., Malykh, Ya. N., Rylova, S. N., and Somova, O. G. (1997) *Biochemistry (Moscow)*, **62**, 557-562.
2. Rylova, S. N., Somova, O. G., and Dyatlovitskaya, E. V. (1998) *Biochemistry (Moscow)*, **63**, 1057-1060.
3. Rylova, S. N., Somova, O. G., Zubova, E. S., Dudnik, L. B., Kogtev, L. S., Kozlov, A. M., Alessenko, A. V., and Dyatlovitskaya, E. V. (1999) *Biochemistry (Moscow)*, **64**, 437-441.
4. Dyatlovitskaya, E. V. (1998) *Biochemistry (Moscow)*, **63**, 55-61.
5. Hannun, Y. A., and Linardic, C. M. (1993) *Biochim. Biophys. Acta*, **1154**, 223-236.
6. Hannun, Y. A. (1994) *J. Biol. Chem.*, **269**, 3125-3128.
7. Rylova, S. N., Kozlov, A. M., Kogtev, L. S., Gaenko, G. P., and Dyatlovitskaya, E. V. (1997) *Biochemistry (Moscow)*, **62**, 1053-1056.
8. Higgins, G. N., and Anderson, R. M. (1931) *Arch. Pathol.*, **12**, 186-202.
9. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.*, **226**, 497-508.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
11. Dyatlovitskaya, E. V. (1995) *Biochemistry (Moscow)*, **60**, 629-634.
12. Kandyba, A. G., Somova, O. G., Kozlov, A. M., Zubova, E. S., Dudnik, L. B., Alessenko, A. V., Shvetz, V. I., and Dyatlovitskaya, E. V. (2000) *Biochemistry (Moscow)*, **65**, 703-706.
13. Dyatlovitskaya, E. V., Lemenovskaya, A. F., Greshnykh, K. P., Ushakov, A. N., and Bergelson, L. D. (1973) *Biokhimiya*, **38**, 943-948.
14. Michel, C., van Echten-Deckert, G., Rother, J., Sandhoff, K., Wang, E., and Merrill, A. H., Jr. (1997) *J. Biol. Chem.*, **272**, 22432-22437.
15. Geeraert, L., Mannaerts, G. P., and Van Veldhoven, P. P. (1997) *Biochem. J.*, **327**, 125-132.
16. Mikami, T., Kashiwagi, M., Tsuchihashi, K., Akino, T., and Gasa, S. (1998) *J. Biochem.*, **123**, 906-911.