EFFECT OF CYCLOPHOSPHAMIDE ON GANGLIOSIDE CONTENT AND PROFILE IN METASTASIZING AND NONMETASTASIZING MOUSE MAMMARY GLAND CARCINOMA

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During growth and metastasization of a tumor considerable changes take place in the structure and properties of the cell membrane [2, 3], which is the site of predominant localization of glycosphingolipids containing sialic acid. Since cells of a metastizing tumor are known to lose their glycocalyx [4], it can be postulated that the level of lipid-bound sialic acids (LSA) and the ganglioside profile in tumors differing in metastatic potential will differ. The few data in the literature on this question are contradictory [5, 6, 8]. The effect of chemotherapeutic agents with marked antitumor and antimetastatic action of the composition of tumor cell gangliosides has not been studied.

To demonstrate any connection between gangliosides and metastasization of a tumor we studied the content and profile of gangliosides in metastasizing and nonmetastasizing mammary gland carcinomas of mice before and after treatment with cyclophosphamide (CP).

EXPERIMENTAL METHOD

Experiments were carried out on male A/Snell mice aged 5 weeks, weighing about 20 g, with a subcutaneously implanted mammary gland carcinoma BMP-II, metastasizing into the liver and ovaries in 100% of cases, and a nonmetastasizing mammary gland carcinoma BMP-0. CP was injected intraperitoneally in a single dose of 150 mg/kg body weight on the 9th day after inoculation of the tumors. The tumors were removed on the 9th, 12th, 15th, 19th, and 23rd days after inoculation. Gangliosides were isolated from tumor tissue, prufied as described previously [1], dissolved in a chloroform–methanol mixture (2:1), and the LSA content was determined by the resorcinol method [7]. The gangliosides were identified by microthin-layer chromatography on silica-gel plates, using reference substances of known structure, and also by investigation of products of enzymic hydrolysis of gangliosides by neuraminidase from a noncholera vibrio [1].

EXPERIMENTAL RESULTS

The LSA levels in the tissues of the mammary gland carcinomas before and after treatment with CP are given in Table 1. During growth of the BMP-0 tumor (9th-20th days after inoculation) the LSA level was virtually unchanged, whereas in BMP-II it rose from 340 to 494 μg/g protein on the 15th day after inoculation, after which it fell gradually. In BMP-0 CP caused a stable increase (by 70-80%) in the LSA level throughout the period of observation, whereas in BMP-II the maximal increase (by 30-40%) occurred on the 15th-18th day after inoculation, after which this parameter fell.

The specimen of gangliosides from carcinomas BMP-II and BMP-0 consisted of six main fractions which were partially characterized by thin-layer chromatography and enzymic hydrolysis as corresponding to N-acetylhematoside (fraction I), N-glycolyhematoside (fraction II), ganglioside G_{M_1} (fraction III), ganglioside $G_{D_{1a}}$ (fraction IV), ganglioside $G_{D_{1b}}$ (fraction V), and ganglioside G_{T_1} (fraction VI). The composition of the gangliosides of carcinomas BMP-II and BMP-0 on different days after inoculation, before and after treatment with CP, is

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TABLE 1. LSA Content (in μg/g protein) in Tumors BMP-0 and BMP-II before and after Treatment with CP

Tumor	Time after inoculation, days	Untreated intact tumors	CP-treated tumors
Mammary gland carcinoma BMP-II	9	340	—
	12	360	420
	15	494	620
	19	415	582
	23	405	448
Mammary gland carcinoma BMP-0	9	390	—
	12	430	760
	15	380	665
	20	420	729

Legend. Mean results of 4-5 experiments given.

TABLE 2. Composition of Gangliosides (in % of total content) of BMP-II and BMP-0 Tumors before and after Treatment with CP

Tumor	Time after inoculation, days	Intact tumors						Tumors treated with CP on 9th day					
		G_{M3}		G_{M1}	G_{D1a}	G_{D1b}	G_{T1}	G_{M3}		G_{M1}	G_{D1a}	G_{D1b}	G_{T1}
		N-Ac	N-Glc					N-Ac	N-Glc				
Mammary gland carcinoma BMP-0	9	4	20	32	36	8	—	—	—	—	—	—	—
	12	7	15	26	31	14	7	3	15	22	29	17	14
	15	4	12	23	35	19	7	3	12	24	28	21	12
	20	2	6	21	44	21	6	—	4	32	32	20	12
Mammary gland carcinoma BMP-II	9	—	8	13	65	11	3	—	—	—	—	—	—
	12	—	8	18	57	14	3	5	11	29	32	14	9
	15	—	9	16	49	18	8	3	8	25	34	17	13
	19	—	7	23	48	15	7	—	9	24	42	19	6

given in Table 2. The greatest differences in the composition of the gangliosides of the intact tumors with different metastatic potential were observed in the early stages of their development. Whereas in BMP-0 the main components of the gangliosides were G_{D1a} and G_{M1} and, to a lesser degree, G_{D1b} and the hematosides, in metastisizing carcinoma BMP-II the main ganglioside on the 9th-15th days after inoculation was G_{D1a} (50-65% of the total gangliosides).

The action of CP on BMP-0 was not followed by any significant changes in the composition of the gangliosides (some decrease in the relative content of G_{D1a} and an increase in the contribution of trisialoganglioside). Conversely, the action of CP on BMP-II led to substantial changes in the composition of the gangliosides during the period of maximal inhibition of metastasization of this tumor. On the 3rd-6th day after injection of the drug (12th-15th day after inoculation) the relative content of G_{D1a} was considerably reduced, whereas the levels of G_{M1} and the hematosides were appreciably higher. On the whole, during this period of observation, the ganglioside composition of the treated BMP-II was identical to the ganglioside composition of the treated BMP-II was identical to the ganglioside profile of the untreated BMP-0 on the 12th-15th day after inoculation. In the later stages after injection of the drug its antimetastatic effect weakened and differences between the treated and intact BMP-II (19th day after inoculation) ceased to be significant.

The results indicate marked differences in ganglioside biosynthesis in tumors with different metastatic potential. This may be due to the fact that the pools of gangliosides that are precursors in biosynthesis of G_{D1a} are considerably increased in the metastasizing carcinoma BMP-II, so that it accumulated predominantely in this tumor.

The action of CP, causing marked inhibition of growth of the nonmetastasizing tumor BMP-0, caused no significant changes in the ganglioside profile, whereas inhibition of metastasization by this drug was accompanied by marked changes in the ganglioside composition of the BMP-II tumor, toward its "increasing complexity."

Taken as a whole, the results obtained by comparing gangliosides of intact BMP-0 and BMP-II tumors and also gangliosides of tumors treated with CP point to an evident connection between the ganglioside profile and the ability of the tumors to metastasize.

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