BIOPHYSICS AND BIOCHEMISTRY

Functional Activity of Sphingomyelin Cycle in Rat Liver in Chronic Toxic Hepatitis

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Activities of sphingomyelinase and ceramidase decreased in the liver in chronic toxic hepatitis and the balance between the levels of proapoptotic ceramide and antiapoptotic sphyngosine-1-phosphate shifts towards the latter substance. Pronounced changes in the qualitative and quantitative composition of fatty acids in the sphingomyelin cycle effector molecules were revealed.

Key Words: *toxic hepatitis; sphingomyelinase; ceramidase; ceramide; sphyngosine-1-phosphate*

A stable trend to chronization of hepatitis of noninfectious origin is observed during recent years [4]. Chronic inflammation is an important risk factor of tumor growth [2]. We studied activities of the key enzymes of the sphingomyelin cycle in the liver in the course of chronic hepatitis and evaluated shifts in the balance between the content of sphingomyelin cycle components, which, as second messengers, transmit the pro- and antiapoptotic signals [11,12].

MATERIALS AND METHODS

The study was carried out on male rats (200-300 g). Group 1 (control) consisted of 15 intact rats. Group 2 consisted of 35 animals in which chronic toxic hepatitis was induced. At stage 1, the rats were injected with 50% oil solution of CCl_4 (3 subcutaneous injections at 4-day intervals; 0.45 ml solution/100 g). Stage 2 started 15 days after the 3rd injection of CCl_4. The animals received subcutaneous injections of 20% CCl_4 (0.1 ml solution/100 g) once a week for 2 months. The rats were examined on days 41, 46, 51, 56, 61, 66, and 71 after the 1st injection of CCl_4. Serum activities of aminotransferases (ALT and AST) and concentrations of total bilirubin were measured and thymol test was carried out using Lachema diagnostic kits. Activity of LPO in the liver was evaluated by the content of TBA-reactive products in the homogenate. Activities of neutral sphingomyelinase (SM) and ceramidase were measured [1,3]. The decrease in the concentration of substrates (sphingomyelin and ceramide) in the incubation mixture was evaluated by chromatography in a thin layer of silica gel. The levels of SM, ceramide, and sphyngosine-1-phosphate (S1-P) in lipid extract from the liver were measured by the same method after Folch. Fatty acids (components of the sphingomyelin cycle) were measured by gas-liquid chromatography on a Tsvet-800 chromatoghraph. Liver homogenate protein was measured by the method of Lowry. Liver morphology was studied by light microscopy on sections stained with hematoxylin and eosin. The significance of differences between the parameters in experimental and control groups was evaluated using nonparametric Mann—Whitney test.

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TABLE 1. Activities of SM and Ceramidase (μmol P_i/min×mg protein), Content of Sphingomyelin, Ceramide, and S1-P (μg/mg protein) in Rat Liver in Chronic Toxic Hepatitis ($X \pm m$)

Parameter	Control	Day of observation						
		41	46	51	56	61	66	71
SM	31.18±1.29	32.93±2.15	28.32±2.01	24.12±1.87*	20.22±1.92*	18.23±1.15*	17.34±1.45*	19.21±2.01*
Ceramidase	3.97±0.26	3.83±0.21	2.91±0.27	2.07±0.18*	1.97±0.13*	1.54±0.11*	1.63±0.09*	1.71±0.15*
Sphingomyelin	14.31±1.13	16.71±1.53	18.34±1.49	21.23±1.85*	23.45±2.12*	37.15±2.76*	34.39±3.01*	36.47±3.32*
Ceramide	5.63±0.34	6.12±0.53	5.11±0.34	3.26±0.29*	2.97±0.23*	2.74±0.21*	2.79±0.23*	2.87±0.27*
S1-P	34.12±2.17	42.17±3.34*	45.43±3.12*	48.92±3.21*	54.33±1.21*	50.21±3.12*	48.22±2.34*	49.70±2.12*

Note. P_i: inorganic phosphate. Here and in Tables 2, 3: *$p<0.05$ compared to the control.

TABLE 2. Fatty Acid Composition of Sphingomyelin, Ceramide, and S1-P Molecules in Rat Liver (% of Total Content of Fatty Acids) in Chronic Toxic Hepatitis ($X \pm m$)

Parameter		Control	Day of observation						
			41	46	51	56	61	66	71
Sphingomyelin	16:0	51.82±3.21	50.38±4.91	49.12±4.71	48.55±4.63	45.11±4.33	40.25±3.95*	38.11±3.75*	35.11±2.92*
	16:1	2.14±0.13	2.87±0.19	3.52±0.27	3.61±0.32*	3.75±0.34*	4.15±0.32*	4.38±0.41*	4.46±0.44*
	18:0	9.35±0.48	9.61±0.87	8.95±0.78	9.35±0.91	9.36±0.92	9.76±0.89	8.87±0.85	9.13±0.91
	18:1	0.98±0.05	0.99±0.08	0.87±0.08	0.82±0.07	1.35±0.11*	1.93±0.18*	2.13±0.21*	2.21±0.21
	18:2	1.12±0.11	1.36±0.12	1.28±0.11	0.73±0.05*	0.42±0.03*	—	—	—
	20:0	3.57±0.26	3.44±0.31	3.35±0.31	2.93±0.22	3.12±0.31	3.84±0.32	3.72±0.29	3.71±0.35
	22:2	7.13±0.56	6.97±0.65	6.85±0.21	6.54±0.63	7.13±0.55	7.22±0.62	6.91±0.62	6.97±0.59
Ceramide	16:0	51.82±3.21	49.12±4.66	54.12±5.15	61.12±5.10*	62.34±6.12*	70.21±6.98*	71.22±7.11*	69.34±6.13*
	16:1	2.13±0.13	2.33±0.21	2.45±0.23	2.55±0.22	2.71±0.24	2.61±0.23	2.43±0.23	2.39±0.20
	18:0	9.34±0.48	9.55±0.91	9.78±0.89	9.40±0.87	8.95±0.86	9.91±0.89	8.97±0.87	9.53±0.78
	18:1	0.95±0.05	1.13±0.11	0.85±0.02	0.94±0.06	0.99±0.08	0.95±0.09	0.89±0.08	0.87±0.07
	18:2	0.17±0.01	0.19±0.01	0.22±0.02	—	—	—	—	—
	20:0	3.56±0.26	3.11±0.31	3.44±0.31	2.99±0.27	3.12±0.29	3.11±0.31	3.57±0.31	3.63±0.32
	22:2	7.17±0.56	5.17±0.43	5.10±0.45	4.95±0.39	4.20±0.37	4.50±0.35	4.47±0.44	4.32±0.39
S1-P	16:0	51.81±3.21	52.22±4.84	53.13±4.98	58.16±4.73*	60.51±5.51*	60.34±5.78*	65.61±6.12*	69.21±6.73*
	16:1	2.13±0.13	2.19±0.17	1.98±0.15	1.87±0.18	1.91±0.19	2.12±0.21	2.15±0.18	2.23±0.22
	18:0	9.32±0.48	9.33±0.92	9.25±0.91	12.15±1.12*	13.11±1.15*	13.14±1.21*	13.51±1.31*	13.72±1.29*
	18:1	0.92±0.05	0.76±0.05	0.99±0.08	1.11±0.11	1.12±0.11	0.97±0.08	0.89±0.06	0.76±0.02
	18:2	0.21±0.01	0.33±0.03	0.27±0.02	—	—	—	—	—
	20:0	3.56±0.26	3.34±0.27	3.76±0.34	3.81±0.37	3.91±0.37	3.76±0.29	3.12±0.29	2.98±0.27
	22:2	7.15±0.56	5.34±0.57*	4.98±0.47*	3.76±0.29*	3.12±0.23*	3.22±0.31*	3.31±0.27*	3.11±0.29*

TABLE 3. Fatty Acid Saturation Coefficient (SC) of Sphingomyelin, Ceramide, and S1-P in Rat Liver in Chronic Toxic Hepatitis ($X \pm m$)

Parameter	Control	Day of observation						
		41	46	51	56	61	66	71
Sphingomyelin SC	5.69±0.43	5.20±0.48	4.91±0.43	5.20±0.46	4.72±0.34	4.21±0.38	3.94±0.29	3.69±0.35
Ceramide SC	6.21±0.54	7.00±0.67	7.81±0.67	8.98±0.79	9.70±0.87*	10.59±1.10*	11.03±0.98*	11.18±1.11*
S1-P SC	6.18±0.61	7.53±0.71	8.11±0.76	11.00±1.12*	12.61±1.12*	12.24±1.18*	12.95±1.10*	13.72±1.12*

RESULTS

By day 41, serum content of total bilirubin in experimental animals was 1.7 higher than in the control and remained 1.2-1.5 times elevated until the end of the experiment ($p<0.05$). Thymol test values were elevated ($p<0.05$) throughout the entire observation period, while serum activities of AST and ALT and concentration of TBA-active products in the liver virtually did not differ from the control. Histological activity index (HAI) was 6.7-7.5 points (*vs.* 1.50±0.13 in the control; $p<0.05$), which attested to weak inflammatory process. Microscopic examination of the liver preparations showed the mesenchymal inflammatory syndrome. The type and direction of shifts in biochemical and morphological parameters indicated the formation of chronic hepatitis [7].

Activities of SM and ceramidase on days 41-46 did not differ from the control, but significantly decreased starting from day 51 to the end of the experiment these (Table 1). Liver content of SM and S1-P increased significantly throughout the entire experiment (Table 1). The dynamics of liver ceramide content exhibited an opposite trend: its level decreased significantly starting from day 51 (Table 1). Reciprocal shifts of ceramide and sphingomyelin content can be explained by inhibition of sphingomyelin hydrolysis due to reduction of SM activity. The dynamics of fatty acid composition in molecules of the sphingomyelin cycle components indicated exhaustion of the long-chain unsaturated fatty acids and increasing content of saturated fatty acids (Table 2). The saturation coefficient (SC) for S1-P and ceramide molecules increased significantly from day 51 to day 56 of observation and remained high until the end of the experiment (Table 3). No appreciable shifts in the sphingomyelin molecule SC were noted.

Our results indicate that significant increase in fatty acid SC in effector molecules of the sphingomyelin cycle (ceramide and S1-P) could appreciably modulate the intensity of their biological effects [5,6,8] and promote chronization of the process. The shift of the balance between proapoptotic ceramide and antiapoptotic S1-P [10,11] towards the latter substance probably suggests that under conditions of chronic hepatitis the ceramide-mediated apoptosis of hepatocytes no longer plays the important role it played at the stage of acute inflammation of the liver [9]. Sphyngosine-1-phosphate stimulates cell proliferation, which is in line with interpretation of active role of chronic inflammation as the risk factor for tissue malignization [2].

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