Activity of Sphingomyelinase in Rat Liver in Acute and Chronic Toxic Hepatitis: Proportion between Peroxidative and Phospholipase Pathways of Lipid Bilayer Modification

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We showed that sphingomyelinase activity in the liver increased only during the acute phase of toxic hepatitis. Peroxidative modification of hepatocyte membrane bilayer prevailed during the acute phase, while after transformation of the process to the chronic phase phospholipase pathway predominated.

Key Words: *sphingomyelinase; toxic hepatitis; phospholipases A_2 and D; lipid peroxidation*

Components of the sphingomyelin cycle mediate transduction of regulatory signals involved in the formation of the response of functional systems of the organisms to a variety of environmental factors [11,14]. The mechanisms of realization of the cell response can considerably differ due to diversity of exogenous factors. Here we studied the dynamics of the ratio of activity of sphingomyelinase (CM), the key enzyme of sphingomyelin cycle, and peroxidative and phospholipase pathways of modification of the lipid bilayer of cell membranes in acute and chronic hepatitis in rats.

MATERIALS AND METHODS

Experiments were carried out on 85 male Wistar rats weighing 200-230 g. For modeling of acute hepatitis in the experimental group (n=50), 50% oil solution of CCl_4 (0.45 ml per 100g body weight) was injected 3 times with 4-day intervals. For transformation of the process into the chronic stage, the injections were resumed after 15 days: CCl_4 (0.1 ml 20% solution per 100 g body weight) was injected once a week for 2 months. The control group consisted of intact rats (n=35). Functional state of the liver was evaluated by serum activities of aminotransferases (AST and ALT), concentration of total bilirubin, and by thymol turbidity tests using Lachema diagnostic tests. Activities of neutral CM in the liver [1], phospholipase A_2 (PL-A_2), and phospholipase D (PL-D) were measured [3]. The decrease in the content of CM substrate in the incubation medium and accumulation of the products of the corresponding phospholipases were evaluated by thin-layer chromatography. Activity of LPO processes in liver homogenate was evaluated by the content of TBA-reactive products. Activities of catalase and SOD were evaluated as described previously [2,6], protein concentration was measured by the method of Lowry. Morphology of the liver was studied by light microscopy after hematoxylin and eosin staining. Significance of the differences between the groups was evaluated using nonparametric Mann—Whitney test.

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TABLE 1. Activities of CM (mmol Pi/min×mg protein), PL-A_2, and PL-D (mmol Pi/min×mg/protein), and Content of Lysophospholipid (% of Total Phospholipids) in Rat Liver in Acute and Chronic Toxic Hepatitis (X±*m*)

Parameter	Control	Acute hepatitis, days			Chronic hepatitis, days						
		day 5	day 10	day 15	day 41	day 46	day 51	day 56	day 61	day 66	day 71
CM	31.18±1.29	41.84±2.56*	45.67±3.11*	43.39±2.17*	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*
PL-A_2	0.13±0.02	2.76±0.21*	3.84±0.26*	2.98±0.21*	0.54±0.04*	0.76±0.06*	0.64±0.05*	0.54±0.04*	0.55±0.04*	0.43±0.30*	0.55±0.04*
PL-D	0.050±0.004	0.12±0.01*	0.17±0.01*	0.18±0.01*	0.11±0.01*	0.14±0.01*	0.15±0.01*	0.13±0.01*	0.16±0.01*	0.13±0.01*	0.11±0.01*
Lisophospholipid	0.17±0.01	0.34±0.02*	0.32±0.03*	0.29±0.02*	0.23±0.02*	0.34±0.03*	0.35±0.03*	0.26±0.02*	0.29±0.02*	0.34±0.03*	0.32±0.03*

Note. Changes in the content of sphingomyelin, lysophosphatodylcholine, and phosphatide acid in the incubation mixture were evaluated by the concentration of inorganic phosphate (Pi) in eluates from the corresponding fractions. *$p<0.05$ compared to the control.

TABLE 2. Content of TBA-Reactive Products (nmol/mg protein), Activities of Catalase and SOD (mmol/min×mg protein) in Rat Liver during Acute and Chronic Hepatitis (X±*m*)

Parameter	Control	Acute hepatitis, days			Chronic hepatitis, days						
		day 5	day 10	day 15	day 41	day 46	day 51	day 56	day 61	day 66	day 71
TBA-reactive products	3.16±0.06	5.76±0.43*	6.76±0.45*	7.89±0.65*	3.43±0.33	3.56±0.35	3.73±0.29	3.42±0.33	3.21±0.32	3.44±0.29	3.01±0.27
Catalase activity	7.2±0.45	11.34±1.11*	13.21±1.24*	13.45±1.29*	8.17±0.83	7.56±0.65	6.12±0.59	4.56±0.34*	3.94±0.29*	3.43±0.31*	3.29±0.27*
SOD activity	4.11±0.25	7.11±0.67*	8.34±0.78*	9.35±0.86*	5.17±0.51	4.34±0.42	3.67±0.35	2.54±0.21*	2.34±0.21*	2.21±0.21*	2.12±0.19*

Note. *$p<0.05$ compared to the control.

RESULTS

Typical acute inflammation in the liver developed on days 5-15 after the first injection of CCl_4, which was seen from high values of the index of histological activity (IHA) attaining the maximum on day 10: 12.5±0.83 *vs*. 1.5±0.13 in the control ($p<0.05$). Serum activity of both aminotransferases increased ($p<0.05$) and the concentration of total bilirubin increased 5-fold ($p<0.05$; peaks on days 5 and 10, respectively). The results of the thymol turbidity test were highly positive ($p<0.05$). Typical signs of transformation of the inflammatory process in the liver into chronic stage were observed on days 41-71 [7]. During this period, IHA varied from 6.00±0.29 to 8.50±0.36 ($p<0.05$ compared to the control). The concentration of total bilirubin and results of thymol turbidity tests 1.5-2.5-fold surpassed the control values ($p<0.05$). CM activity in the liver during the acute phase significantly increased and peaked on day 10. Transformation of the process into the chronic stage was accompanied by normalization of enzyme activity (Table 1). LPO processes in the liver were considerably activated during the acute phase despite activation of catalase and SOD (Table 2). Chronization of hepatitis was accompanied by normalization of the level of TBA-reactive products starting from day 41 of observation and activity of catalase and SOD (Table 2). Both phospholipases in rat liver were activated during the acute phase of hepatitis, but the major contribution was made by PL-A_2. Chronization of hepatitis had no effect on the ratio of phospholipase activities (Table 1).

Our results agree with the previous data on enhanced TNF-α production during oxidative stress and on direct correlation between CM activity and accumulation of LPO products in the liver *in vivo* [5,10]. It is also known that a close relationship exists between CM activity in the liver and functional state of the thyroid gland [1], while activity of the thyroid gland, in turn, is affected by the state of the liver executing extrathyroid conversion (deiodination) of thyroxin [8]. Hepatotropic toxins negatively affect the thyroid gland due to both direct toxic effects and indirectly (by disturbing thyroxin conversion in the liver) [8]. Our experiments showed that stimulation of CM was observed only during the acute period of the disease, which agrees with published data. Predominance of PL-A_2 activity was characteristic of acute stage of hepatitis accompanied by maximum stimulation of LPO, which also agrees with the capacity of TNF-α to activate both phospholipases [12] and with the fact that their products can act as intracellular activators of CM [9,13]. Our results attest to complex interrelationships between CM activity, degree of activation of the studied phospholipases, and the content of lysophospholipids. This can be explained by differences in fatty acid composition of lysophospholipids formed in different phases of the pathological process, which probably can alter the sign of their modulating influence on CM activity [4]. Free-radical mechanism of the cytotoxic effect of CCl_4 determined the prevalence of peroxidative pathway of modification of hepatocyte membranes during the acute phase of hepatitis. At the same time, activation of PL-A_2 is secondary by its nature. Under conditions of chronization of the pathological process, the phospholipase pathway of modification of the lipid bilayer of cell membranes became predominant.

REFERENCES

1. N. A. Babenko, *Biokhimiya*, **56**, No. 2, 346-353 (1991).
2. O. S. Brusov, A. M. Gerasimov, and L. F. Panchenko, *Byull. Eksp. Biol. Med*., **81**, No. 1, 33-35 (1976).
3. O. N. Vagina, M. E. Zamaraeva, S. V. Gevantmacher, *et al., Biokhimiya*, **60**, No. 10, 1596-1599 (1995).
4. G. G. Gribanov, *Vopr. Med. Khimii*, **37**, No. 4, 2-10 (1991).
5. U. A. Gutner, L. B. Dudnik, V. G. Korobko, and A. V. Alesenko, *Zh. Nevrol. Psikhiatr*., **105**, No. 4, 48-54 (2005).
6. M. A. Korolyuk and L. I. Ivanova, *Lab. Delo,* No. 1, 16-18 (1988).
7. B. N. Matyushin, A. S. Loginov, G. N. Yakimchuk, and V. D. Tkachev, *Vopr. Med. Khimii*, No. 4, 54-56 (1995).
8. Ya. Kh. Turakulov, T. P. Tashkhodzhaeva, and G. M. Artykbaeva, *Probl. Endokrinol*., **37**, No. 4, 44-46 (1991).
9. N. Andrieu-Abaddie and T. Lavade, *Biochim. Biophys. Acta*., **30**, No. 3, 126-134 (2002).
10. C. Y. Chen, Y. L. Huang, and T. H. Lin, *Arch. Biochem. Biophys*., **356**, No. 2, 127-132 (1998).
11. Y. A. Hannun and L. M. Obeid, *Trends Biochem. Sci*., **20**, No. 2, 73-77 (1995).
12. M. Liscovitch, *Ibid*., **17**, No. 10, 393-399 (1992).
13. T. Neitcheva and D. Peeva, *Int. J. Biochem. Cell Biol*., **27**, No. 10, 995-1001 (1995).
14. Y. Sawai and Y. A. Hannun, *Chem. Phys. Lipids*. **102**, Nos. 1-2, 141-147 (1999).