Effect of Phospholipid Hepatoprotectors on the Course of Experimental Reye's Syndrome

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Hepatoprotectors containing phospholipids exert a therapeutic effect in experimental Reye's syndrome in rats induced by 4-pentenoic acid. Eplir more effectively than essentiale decreased necrosis of the liver, reduced serum enzymes and bilirubin, alleviated hypoglycemia, suppressed MDA production, and improved ketogenesis and detoxication of ammonium and phenols.

Key Words: *Reye's syndrome; 4-pentenoic acid; eplir; essentiale; liver*

Reye's syndrome (RS) is a complication of salicylate therapy of viral infections (B influenza, varicella) in children aged 4-16 years [6]. Clinical course of RS is characterized by acute encephalopathy and fatty degeneration of the liver and other viscera. The mortality is 30-70% [4,7]. Carnitine deficiency and mitochondrial injury with impairment of bioenergetics, β-oxidation of long- and medium-chain fatty acids, and urea synthesis are the main pathogenetic factors [4,8]. Despite early diagnosis and resuscitation measures, the mortality from RS remains about 10-30% [11].

Hepatoprotective agents improving bioenergetics, lipid metabolism, and antitoxic function of the liver seem to be promising in the treatment of RS. We investigated the effect of the original hepatoprotector eplir (a complex of phospholipids, sulfolipids, tetraterpenoid pigments of sapropel) [5] on structural and metabolic manifestations of experimental RS induced by poisoning with 4-pentenoic (allylacetic) acid which stimulates carnitine acetylation and excretion with urine and impairs β-oxidation of fatty acids [12]. The reference drug was essentiale.

MATERIALS AND METHODS

Experiments were performed on 100 male albino rats weighing 180-200 g kept under standard vivarium conditions. The animals were daily injected (intraperitoneally) with 4-pentenoic acid (ISN) in a dose of 20 mg/kg for 7 days. On days 8-30 of the experiment they were intragastrally treated with eplir (30 mg/kg) in 1% starch suspension or essentiale (80 mg/kg, Rhone-Poulenc Rorer). Controls were given distilled water or starch. The rats were decapitated under ether narcosis 12 h after the last dose of hepatoprotectors or solvents. Activities of alanine and aspartate aminotransferase, acid and alkaline phosphatases, γ-glutamyl transpeptidase (GTP), and the content of bilirubin, lipids, ketone bodies (acetone, β-hydroxybutyric acid), glucose, protein, ammonium, phenols [2], and MDA [1] in the blood and the content of ketone bodies [9] and ammonium in liver homogenate [10] were measured. Activities of SDH, β-hydroxybutyrate dehydrogenase, acid phosphatase and the content of RNA, lipids, and glycogen in cryostat sections of the liver were measured histochemically with subsequent cytophotometry [3]. The data were processed using parametrical Student's *t* test.

RESULTS

After 7 injections of 4-pentenoic acid (experimental RS) the mortality was 20%. Serum transaminases, acid phosphatase, GTP activities indicating hepatocyte cytolysis increased by 3.0-4.8 times. Cholestasis was accompanied by a 3.3-fold increase of alkaline phos-

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phatase activity, the contents of total and indirect bilirubin increased 1.5- and 5.6-fold, respectively. All animals developed hyperlipidemia, hypoglycemia, and hypoproteinemia, the content of acetone and β-hydroxybutyric acid decreased. The content of ammonium and phenols increased 4.5 times, MDA 3.5 times, and the level of urea dropped by half. Analysis of liver homogenates showed deficiency of ketone bodies and accumulation of ammonium (Table 1). Morphohistochemical analysis of the liver showed structural changes in hepatic laminae, circulatory disorders in the sinusoid system, zonal necroses, protein and fatty degeneration of the parenchyma. The content of RNA and activities of mitochondrial enzymes (SDH, β-hydroxybutyrate dehydrogenase) decreased, glycogen disappeared, and activity of lysosome marker acid phosphatase increased.

In the controls, the mortality reached 40% during 30 days. Serum enzyme activities increased by 21-37% more, bilirubin content increased by 45%, the content of lipids and ammonium increased by 1.5 times and phenols by 1.7 times, the content of ketone bodies, glucose, protein, and urea decreased, and the production of MDA was stabilized. Disturbances in ketogenesis and ammonium detoxication in the liver progressed, massive hepatocyte necroses developed, the content of RNA and mitochondrial enzyme activities decreased, and acid phosphatase activity was further increased (Table 1).

Hepatoprotectors containing phospholipids exerted a therapeutic effect in experimental RS, causing regression of structural and metabolic disorders in the liver. All animals treated with eplir and essentiale remained alive until the end of experiment. Hepatoprotector therapy decreased 1.5-4.3 times serum activities of hepatic enzymes, lowered the content of bilirubin, lipids, ammonium, phenols, and MDA; the content of ketone bodies, glucose, protein, and urea increased

TABLE 1. Effects of Eplir and Essentiale on Biochemical Parameters of Rat Blood Serum and Liver Homogenates after Intoxication with 4-Pentenoic Acid (*M±m*, *n*=10)

Parameter	Intact animals	Experimental RS			
		7 days	30 days		
			control	+eplir	+essentiale
Blood serum, per 1 liter:					
SGPT, μmol	0.51±0.01	2.08±0.06	2.83±0.07*	0.75±0.02*+°	1.00±0.04*+
SGOT, μmol	0.65±0.01	1.92±0.05	2.32±0.04*	0.92±0.02*+°	1.28±0.02*+
Acid phosphatase, U	10.7±1.4	45.5±1.2	61.3±2.5*	14.4±1.7*+°	20.3±1.1*+
Alkaline phosphatase, U	223.9±3.4	737.2±9.7	998.3±9.1*	265.5±9.3*+°	386.4±7.5*+
GTP, μmol	0.25±0.01	1.20±0.05	1.64±0.08*	0.44±0.04*+°	0.80±0.04*+
Bilirubin, μmol:					
total	12.2±0.6	18.8±0.5	27.3±1.5*	14.4±0.9*+°	16.6±0.6*+
indirect	1.2±0.1	6.7±0.2	9.5±0.3*	1.4±0.1*+°	2.1±0.1*+
Lipids, g	2.2±0.1	5.1±0.2	7.8±0.3*	2.3±0.1*+°	3.4±0.1*+
Acetone, μmol	155.0±4.5	77.5±2.7	50.4±1.9*	127.0±2.6*+	108.4±2.3*+
β-Hydroxybutyric acid, μmol	186.4±4.6	62.4±1.9	36.6±1.8*	161.7±2.7*+	148.4±2.4*+
Glucose, mmol	6.50±0.12	3.31±0.15	2.19±0.17*	6.14±0.19*+	5.93±0.17*+
Protein, g	80.0±3.5	45.4±2.0	38.9±1.9*	69.2±1.2*+°	62.3±1.5*+
Ammonium, μmol	41.1±2.1	181.3±12.2	272.7±9.5*	62.1±3.2*+°	91.0±2.9*+
Urea, mmol	8.3±0.3	4.1±0.1	3.0±0.1*	6.3±0.2*+°	5.6±0.1*+
Phenols, μmol	60.5±3.3	270.4±8.0	470.6±9.5*	94.8±6.4*+°	45.0±8.6*+
MDA, mmol	1.45±0.05	5.12±0.26	4.94±0.14*	1.86±0.12*+	2.21±0.17*+
Liver homogenate, per 100 g:					
Acetone, μmol	64.5±1.2	29.9±1.0	22.7±1.2*	56.2±1.3*+°	47.5±1.0*+
β-Hydroxybutyric acid, μmol	52.3±1.2	15.8±0.6	10.2±1.0*	47.3±1.4*+°	41.2±1.4*+
Ammonium, μmol	5.6±0.2	27.9±0.7	35.7±1.0*	10.2±0.5*+°	13.7±0.9*+

Note. $p<0.05$: *vs.* RS, +*vs.* control (starch gel), °*vs.* essentiale. All differences are reliable between: RS and intact animals, controls and intact animals, essentiale+RS group and control.

1.8-4.0 times. Production of ketone bodies in the liver increased, the content of ammonium decreased, normal liver histoarchitectonics was restored, hemodynamic disorders disappeared, no necroses and hepatocyte degeneration were seen, glycogen appeared, RNA content and activities of mitochondrial and lysosomal enzymes returned to normal. Eplir showed a higher therapeutic activity than essentiale by the majority of biochemical and histochemical parameters (Table 1).

Hence, phospholipid hepatoprotectors eplir and essentiale, effective antioxidants and substitute drugs improving the barrier and matrix function of hepatocyte membranes [5], repaired liver structure and improved metabolism in experimental RS. The effect of hepatoprotectors can be regarded as pathogenetic. Hyperlipidemia and increased content of ketone bodies probably result from activation of β-oxidation of medium- and long-chain fatty acids in mitochondria. Ketone bodies were utilized as glycogenesis substrates, which led to correction of hypoglycemia. Hepatoprotectors improved the antitoxic function of the liver by stimulating bilirubin glucuronidation, phenol neutralization, and ammonium utilization in the urea. The latter effect might be due to elimination of the inhibitory effect of fatty acids on urea synthesis enzymes [4,8].

Experimental data indicate that hepatoprotectors containing phospholipids can be added to combined therapy of RS.

REFERENCES

1. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
2. V. G. Kolb and V. S. Kamyshnikov, *Handbook of Clinical Chemistry* [in Russian], Minsk (1982).
3. Z. Loida, R. Gossrau, and T. Shibler, *Histochemistry of Enzymes* [in Russian], Moscow (1982).
4. S. A. Mal'mberg, A. Z. Chebysheva, N. V. Semenova, and V. I. Shelkovskii, *Pediatriya,* No. 3, 92-94 (1995).
5. A. S. Saratikov and A. I. Vengerovskii, *Eksp. Klin. Farmakol.,* No. 1, 8-11 (1995).
6. E. Claas, A. Osterhaus, and R. van Beek, *Lancet,* **351,** No. 9501, 472-477 (1998).
7. S. Larsen, *Med. Sci. Law,* **37,** No. 3, 235-241 (1997).
8. J. Lemasters, A. Nieminen, T. Qian, *et al., Biochim. Biophys. Acta,* **1366,** No. 1-2, 177-196 (1998).
9. H. Okuda, J. Kawashima, and J. Tokushima, *J. Exp. Med.,* **12,** No. 1, 11-22 (1965).
10. V. Peden, *J. Lab. Clin. Med.,* **63,** No. 3, 332-335 (1964).
11. R. Perez, M. Vasquez, C. Martinez-Pardo, *et al., An. Esp. Pediatr.,* **46,** No. 5, 460-463 (1997).
12. T. Sugimoto, M. Woo, N. Nishida, *et al., Brain Res.,* **12,** No. 4, 417-422 (1990).