METHODS

Experimental Models of Reye's Syndrome

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 129, No. 4, pp. 475-477, April, 2000
Original article submitted August 30, 1999

Intraperitoneal administration of 4-pentenoic acid (20 mg/kg/day for seven days or ten doses of 50 mg/kg with 4 h intervals) or acetylsalicylic acid (50 mg/kg 1 hour after single pyrogenal injection) to rats reproduced metabolic disorders characteristic of Reye's syndrome: hyperenzymemia, hyperbilirubinemia, decreased levels of ketone bodies and urea, hypoglycemia, hypoproteinemia, accumulation of ammonia and phenol, and acidosis. Necroses and microvesicular steatosis were found in the liver.

Key Words: *experimental models of Reye's syndrome; 4-pentenoic acid; acetylsalicylic acid; pyrogenal*

Reye's syndrome (RS) characterized by acute encephalopathy and lipid degeneration of the liver appears in children treated with salicylates for influenza B, varicella, and other viral infections and without treatment causes 30-70% mortality [4,5]. In RS, salicylates and viral antigens damage mitochondria, which causes bioenergetic disorders and impairs β-oxidation of long- and medium-chain fatty acids and urea synthesis [7]. Two experimental models of RS in rats were described: poisoning with 4-pentenoic acid (4-PA) or acetylsalicylic acid with simultaneous administration of bacterial lipopolysaccharides (LPS). In these experiments, 4-PA (allylacetic acid) stimulated carnitine acylation and its excretion with urine, increased the concentration of ammonia in the blood, and caused mitochondrial swelling and microvesicular steatosis of the liver [10,12]. Acetylsalicylic acid administered in combination with LPS reduced the ATP/ADP ratio, decreased the levels of ketone bodies and acetylcoenzyme A and induced accumulation of long- and medium-chain fatty acids [6].

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This paper describes metabolic disorders typical of RS observed in experimental models of this severe complication.

MATERIALS AND METHODS

Experiments were performed in winter on 100 male outbred albino rats weighing 180-200 g. The rats were kept under of natural light/dark regimen and had free access to standard food and water. Group 1 rats received intraperitoneal injections of 4-PA (ISN) in a dose of 20 mg/kg daily for seven days [10]. Group 2 rats received 10 injections of 50 mg/kg 4-PA at 4 h intervals [12]. Group 3 rats received Aspisol (acetylsalicylic acid for injection, Bayer) in a dose 50 mg/kg 1 h after single administration of pyrogenal (0.2 mg/kg) [6].

The rats were decapitated under ether anesthesia 12 h after the last injection. Blood serum was sampled for determination of alanine and aspartate aminotransferases, acid and alkaline phosphatases, and γ-glutamyltranspeptidase activities, bilirubin, protein, urea, phenol, lipids, glucose [2], and MDA [1]. The content of ammonia [8] and ketone bodies (acetone and β-hydroxybutyric acid) was measured in the blood and liver homogenates. Blood pH was measured. Activi-

0007-4888/00/0004-400$25.00

ties of certain organelle-specific ezymes and the content of lipids and glycogen [3] were determined in cryostat sections of the liver. The data were processed by Student's *t* test.

RESULTS

Animal mortality in group 1 was 20%. Biochemical indices of blood serum attested to severe metabolic disorders in the liver. Transaminases, acid phosphatase, and γ-glutamyltransferase activities, markers of hepatocyte lysis, increased 3.0-4.8-fold. The rats displayed signs of cholestasis with a 3.3-fold elevation of alkaline phosphatase activity and 1.5- and 5.6-fold increases in total and indirect bilirubin, respectively. The bilirubin glucuronidation coefficient (the ratio of glucuronidated to total bilirubin) decreased to 64% (*vs.* 90% in normal). Other findings were hypoproteinemia, hyperlipidemia, decreased content of ketone bodies, and hypoglycemia. The content of ammonia and phenol increased 4.5-fold and MDA 3.5-fold, while the concentration of urea decreased 2-fold. The ammonia nitrogen fraction in the total nonprotein nitrogen fraction (ammonia+urea) increased from 0.5% (normal level) to 4.2% (Table 1).

Mortality in group 2 rats was 25%. The increase in transaminase, acid phosphatase activities and MDA content, as well as hypoproteinemia, hyperlipidemia, and hypoglycemia in this group were less pronounced than in group 1. Similarly, there was a smaller decrease in the level of ketone bodies in blood serum. Cholestasis and accumulation of toxic products of protein metabolism were more pronounced. Alkaline phosphatase activity increased 3.9-fold, and the concentration of total and indirect bilirubin increased 2.3- and 11.5-fold, respectively. The coefficient of bilirubin glucuronidation decreased to 49%, the levels of ammonia and phenol increased 5.4- and 6.7-fold, respectively, and the content of urea decreased 1.5-fold. The fraction of ammonia nitrogen in total nonprotein nitrogen reached 3.8% (Table 1).

Group 3 displayed the highest mortality rate (40%); however, hyperenzymemia, hypoproteinemia, and especially MDA production were less pronounced than in rats treated with 4-PA. The content of total serum bilirubin and indirect bilirubin increased 1.6-

TABLE 1. Biochemical Parameters of Rat Blood and Liver Homogenates in Experimental RS (*M*±*m*, *n*=8)

Parameter	Intact rats	4-PA, mg/kg		Acetylsalicylic acid+pyrogenal
		20	50	
Blood serum, per liter				
Alanine aminotransferase, μcat	0.51±0.01	2.08±0.06*	1.73±0.05*+	1.56±0.04*+°
Aspartate aminotransferase, μcat	0.65±0.01	1.95±0.05*	1.56±0.04*+	1.44±0.02*+°
Acid phosphatase, U	10.7±1.4	45.3±1.2*	38.0±1.8*+	32.1±1.2*+°
Alkaline phosphatase, U	223.9±3.4	737.2±9.7*	864.7±20.6*+	698.2±10.7*+°
γ-Glutamyl transpeptidase, μcat	0.25±0.01	1.20±0.05*	1.08±0.05*	0.87±0.04*+°
Bilirubin, μmol total	12.2±0.6	18.8±0.5*	27.5±0.8*+	19.3±0.4*°
indirect	1.2±0.1	6.7±0.2*	13.9±0.2*+	9.5±0.1*+°
Protein, g	80.0±3.5	45.0±2.0*	61.5±1.1*+	67.1±1.7*+°
Ammonia, mmol	0.041±0.002	0.181±0.012*	0.223±0.020*+	0.313±0.021*+°
Urea, mmol	8.3±0.2	4.1±0.1*	5.5±0.4*+	5.2±0.2*+
Phenol, μmol	60.5±3.3	270.4±8.0*	408.2±10.6*+	486.8±8.6*+°
Lipids, g	2.2±0.2	5.1±0.1*	4.7±0.1*+	2.4±0.2+°
Acetone, μmol	155.0±4.5	77.5±2.7*	104.3±3.1*+	80.3±3.8*°
β-Hydroxybutyric acid, μmol	186.4±4.6	62.4±1.9*	91.4±2.2*+	69.4±2.7*°
Glucose, mmol	6.5±0.1	3.3±0.1*	4.3±0.2*+	3.5±0.1*°
MDA, mmol	1.45±0.05	5.12±0.26*	4.42±0.09*+	1.95±0.09*+°
Liver homogenate, per 100 g				
Ammonia, μmol	5.2±0.3	28.0±0.9*	32.2±1.1*+	37.6±1.2*+°
Acetone, μmol	64.5±2.1	27.8±1.9*	40.6±1.9*+	30.2±1.9*°
β-Hydroxybutyric acid, μmol	52.3±1.3	19.4±1.7*	33.2±1.9*+	23.7±1.9*°

Note. $p < 0.05$: *in comparison to intact rats; +in comparison to 4-PA, 20 mg/kg; °in comparison to 4-PA, 50 mg/kg

and 8-fold, while bilirubin glucuronidation decreased to 50%. Lipids remained at a nearly normal level. The levels of ketone bodies, glucose, and urea decreased 1.6-2.7-fold. The accumulation of abnormal products of protein metabolism was most pronounced: the content of ammonia and phenol increased 7.6- and 8-fold, respectively, and the fraction of ammonia in the nonprotein nitrogen fraction increased to 5.6% (Table 1).

In all groups, blood pH decreased to 7.23-7.27. Low levels of ketone bodies and accumulation of ammonia were found in liver homogenates. Ketogenesis was most markedly inhibited in groups 1 and 3, whereas the highest level of ammonia was found in group 3 (Table 1). Histological examination of the liver found dyscomplexation of hepatic laminae, focal hepatocyte necrosis, and microvesicular steatosis. Activities of mitochondrial enzymes (β-hydroxybutyrate dehydrogenase and succinate dehydrogenase) decreased, while activity of acid phosphatase (lysosomal marker enzyme) increased, and glycogen disappeared.

Thus, intoxication with 4-PA or acetylsalicylic acid after administration of bacterial LPS reproduces metabolic disorders characteristic of RS. 4-PA causes carnitine deficiency, which impairs transport to mitochondria and β-oxidation of medium- and long-chain fatty acids [10]. Acetylsalicylic acid activates LPO and directly damages mitochondrial membranes; this increases Ca^{2+} uptake, uncouples oxidative phosphorylation, and inhibits β-oxidation [11]. In these RS models, the formation of ketone bodies (products of β-oxidation) decreased; glyconeogenesis from ketone bodies, urea synthesis, and detoxification of phenol and bilirubin were impaired; and the rats displayed hyperenzymemia, acidosis, and necrosis with microvesicular steatosis of the liver.

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