An animal model of fulminant hepatic failure in the rat.

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Abstract

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KEYWORDS: fulminant hepatic failure, brain edema, massive liver injury, hepatic encephalopathy
AN ANIMAL MODEL OF FULMINANT HEPATIC FAILURE IN THE RAT

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Abstract. A reproducible animal model of fulminant hepatic failure was developed by intraperitoneal administration of D-galactosamine hydrochloride to Sprague-Dawley rats. Biochemical and morphological hepatic injury and brain edema resembled human fulminant hepatic failure. This model would facilitate further studies of the pathogenesis of brain dysfunction and evaluation of treatment in fulminant hepatic failure.

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The pathogenesis and treatment of fulminant hepatic failure have been studied experimentally (1–3), although previously reported animal models have not been satisfactory with respect to the requirements for an appropriate model (4). The use of D-galactosamine hydrochloride (GalN) to produce hepatitis similar to human viral hepatitis in laboratory animals was introduced by Decker and Keppler (5). Recently this selective hepatotoxin was used for obtaining a model of fulminant hepatic failure in rabbits (6). Recent observations on the abnormalities of neutral amino acid contents in cerebrospinal fluid of patients with hepatic encephalopathy (7) prompted us to investigate neutral amino acid influx into the brain through the blood-brain barrier in fulminant hepatic failure. A suitable model of fulminant hepatic failure created by administering GalN to rats was characterized in the present study, and physiochemical alterations in the brain are discussed with respect to cerebral function in hepatic failure.

MATERIALS AND METHODS

Materials. GalN was obtained from Sigma Chemical Co., St. Louis, Mo. Other reagents were purchased from sources reported previously (7, 8, 12).

Animals. Male Sprague-Dawley rats weighing 180–220 g were used. Animals were starved overnight prior to the experiments. GalN was administered intraperitoneally as a neutral solution in a single dose of 130 mg/100 g body weight. The animals were given only water ad libitum and sacrificed 24 or 48 h after
GalN injection. Control animals were similarly treated after receiving saline injection. Each test group consisted of 3 rats.

Analytical procedures. Serum glutamic pyruvic transaminase (GPT) activity and serum bilirubin, cholesterol, blood ammonia and plasma glucagon concentrations were determined according to routine laboratory methods (8). Blood ammonia levels were measured according to the method of Fujii and Okuda (9). Prothrombin time, thrombotest and heparplastintest were carried out using citrated blood according to Kleiner (10) and Owren (11). Quantitative determinations of serum amino acids were carried out on a Nihon-Denshi JCL-6AH amino acid analyzer as described previously (12). The progression of rats into hepatic coma was graded into 4 stages (Alert, Drowsy, Stuporous and Comatose) on the basis of the lighting reflex and the response to stimuli. Electroencephalographic recording was carried out as follows: frontoparietal screw type electrodes were placed through the skull on each side into contact with the meninges 3 days prior to hepatotoxin administration and an electrode connector was cemented into place. Recordings were made on a model 1A71 polygraph (San-Ei Electroencephalograph) with a preamplifier sensitivity of 25 μV per cm at a speed of 30 mm per sec. The liver and brain were fixed in 10% buffered formalin and stained with hematoxylin and eosin. Brain dry weight and electrolytes were measured in samples taken from the cortical area after the samples had been blotted to remove cerebrospinal fluid and blood, as previously reported (13).

RESULTS

The relative weight of the liver in GalN-treated rats had increased 24 h after the intraperitoneal injection (Table 1). Serum GPT activity and bilirubin concentration rose significantly on an average to 2200 IU and to 2.9 mg/dl, respectively. Blood ammonia and plasma glucagon concentrations were also markedly elevated at this period of the experiment. Serum cholesterol decreased significantly to a mean level of 46 mg/dl. Heparplastintest and thrombotest values decreased markedly and the prothrombin time became prolonged (Table 2). The serum concentrations of all amino acids including methionine and aromatic amino acids such as phenylalanine and tyrosine but not including histidine or arginine increased to various extents in GalN-treated rats (Table 3).
Abnormal aminograms were also found in other tissues. Fig. 1 shows the elevated levels of neutral amino acids in the brain, liver and muscle from GalN-treated rats. The dry weight of the brain (expressed as a percentage of the wet weight) was 21.0% in GalN-treated rats and 23.0% in the control. The sodium-potassium ratio rose significantly in liver-injured animals (Table 4). The electroencephalogram showed high amplitude slow waves with triphasic-like patterns (Fig. 2). In some of the animals, there was even greater disorganization with further slowing and diminution in amplitude. The liver had extensive
Fig. 1. Aminograms of various tissues in control and GalN-treated rats. Tissue aminograms were determined 24h after GalN administration. One gram of brain, liver or muscle was minced thoroughly and homogenized in 4 volumes of 1% picric acid. The supernatants were used for measuring their aminograms as reported previously (12). Control rats, and GalN-treated rats, •——•. Vertical bars indicate standard error of the mean.
hepatic necrosis microscopically (Fig. 3a). There were diffuse degenerative changes including karyorrhexis and focal coagulation necrosis with many eosinophilic bodies. The surface of the brain appeared diffusely edematous. Increased amounts of perivascular and pericellular spaces were found microscopically in the brain, particularly in the white matter, of the treated animals (Fig. 3b). Decreased staining of sections with hematoxylin and eosin was marked in the subcortical area. These macroscopic and microscopic observations as well as the biochemical data described above were evidence of brain edema in GalN-treated rats.

Fig. 2. Electroencephalographic findings in GalN-treated rats. Recordings were carried out 6, 12 and 24 h after GalN injection.
DISCUSSION

Cerebral function in hepatic encephalopathy has not been well investigated by hepatologists or neurologists. Lack of suitable experimental models may have been part of the problem. The animal model described here showed massive hepatic necrosis and abnormalities both in the electroencephalograms and in the water-mineral content of the brain. The microscopic findings of the brain in this model also suggested cerebral edema. These results are similar to those found in hepatitis patients with massive hepatic necrosis, encephalopathy and cerebral edema. The electroencephalogram in GalN-treated rats showed marked slow wave activity with high amplitude. These findings are frequently seen in human fulminant hepatic failure.

The increased levels of serum free amino acids in GalN-treated rats were similar to laboratory findings in patients with typical fulminant hepatitis (12). These abnormal aminograms also occur in the brain of encephalopathic dogs with a portacaval shunt (14). The marked increases in aromatic amino acids and methionine in human serum over those of branched chain amino acids were accompanied by increased concentrations of tyrosine, phenylalanine and methionine in the cerebrospinal fluid (15). The cerebral content of aromatic amino acids
acids and methionine increased in this model, indicating increased transport of these amino acids through the blood–brain barrier in animals with liver injury (16). Therefore, an imbalance in serum neutral amino acids might lead to an increase in aromatic amino acids and methionine in the brain, supplying their metabolites as possible false neurotransmitters (17).

Cerebral edema in fulminant hepatitis is thought to develop as a direct consequence of metabolic disturbances within the brain. This might be related to severely impaired function of the liver. Water inflow into the brain is due to the influx of sodium and chloride from the extracellular into intracellular compartment. However, little information is available on quantitative cerebral water and electrolyte impairments in fulminant hepatic failure.

Increased cerebral water and Na" content, together with microscopical findings of brain edema were observed in GalN-treated rats. This model is therefore compatible with biochemical and morphological edema of the brain. It is not known, however, whether the progression of hepatic encephalopathy and the development of cerebral edema are associated.

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REFERENCES