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A Novel Model of Acute Hepatic Failure in Dogs With Implications for Transplantation Research

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IN THE LAST few years, liver transplantation has been indicated as therapy for patients with acute hepatic failure.[1] Since liver transplantation represents the last therapeutic option for this entity, it is extremely important to define the timing for the operation based on the evolution of the clinical parameters of the disease.

On the basis of studies conducted on an acetaminophen-induced model of hepatotoxicity in large animals that we previously described,[2] we have defined a new model for acute hepatic failure that lends itself to this type of study.

MATERIALS AND METHODS

Animals

Sixty-two beagles (body weight [BW] ranging from 9 to 14 kg) were purchased from Russel B. Hutton, St Thomas, PA. The dogs were housed in an accredited large animal care facility.

Fifty-two dogs received a total of three subcutaneous injections of acetaminophen in DMSO at a concentration of 600 mg/mL. The first injection of acetaminophen (750 mg/kg BW) was administered at noon; a second injection (200 mg/kg BW) was administered nine hours later; a third dose (200 mg/kg BW) was administered 24 hours after the initial dose. Ten animals were used as controls and they received the same three dosages of DMSO (without acetaminophen).

Biochemical Determinations

Blood levels of acetaminophen. Five animals were used to determine blood acetaminophen levels.[3] A blood sample was taken from each of these animals every four hours beginning with the initial administration of acetaminophen and continuing for 48 hours.

Biochemical parameters. Routine parameters for the evaluation of liver function (Table 1) were performed using standard laboratory tests.

Histology. All nonsurviving dogs underwent full necropsy, which included histologic evaluation of the liver and kidney. Tissues were fixed in 10% neutral buffered formalin, sectioned at 6 μm and stained with hematoxylin and eosin. Histologic examinations were performed in surviving dogs at the time of death.

Statistical analysis. Statistical analyses were performed using a one-way analysis of variance program in the SPCC/PC statistical software (SPSS, Inc, Chicago) package on an IBM-AT microcomputer.

RESULTS AND DISCUSSION

Figure 1 depicts the survival rate, transaminase levels, and acetaminophen levels in the treated dogs. No deaths occurred in the first 24 hours, and only a 10% mortality was observed in the next 24 hours. From 48 through 72 hours, a progressive increase in mortality was observed that reached a level of 90% at 72 hours. A striking increase in the level of serum transaminase levels was achieved between 48 and 60 hours after initial administration of the acetaminophen. The acetaminophen blood level increased for 12 hours, at which point it began to decline slowly over the next 12 hours. Thus, an acetaminophen level >140 μg/mL was achieved for a period of time that ranged from four to 20 hours after initial drug administration.

In treated dogs, histologic examination of the livers obtained at autopsy or at death demonstrated severe zone (centrilobular)

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Table 1. Serum Glutamic-Pyruvic Transaminase, Cholesterol, Albumin, Ammonia, Urea-N, Aromatic Amino Acids (BCAA and AAA) in Dogs (Surviving and Nonsurviving) at Different Times After Intoxication

	0 h	24 h	48 h	60 h	72 h	96 h	120 h
Nonsurviving							
SGPT (U/L)	41 ± 4	75 ± 5	8,206* ± 3,000	21,253*† ± 3,746			
Cholesterol (mg/100 ml)	108 ± 14	91 ± 9	66* ± 22	54*† ± 18			
Albumin (g/100 ml)	3 ± 0.2	2 ± 0.4	1.9* ± 0.4	1.8* ± 0.3			
Ammonia (umole/1)			304* ± 161	525* ± 133			
Urea (N) (mg/100 ml)	26.6 ± 2.8	12* ± 2.1	6.4*† ± 1.9				
AAA (umole/100 ml)	16 ± 2			69.4*† ± 3.1			
BCAA (umole/100 ml)	50.8 ± 3.5			61.4* ± 2.7			
BCAA/AAA	3.14 ± 0.23			0.88*† ± 0.08			
Surviving							
SGPT (ALT) (U/L)	51 ± 61	71 ± 16	642* ± 163	932* ± 432	1,398 ± 603	907* ± 717	
Cholesterol	90 ± 1	83 ± 7	72 ± 20	92 ± 28	85 ± 43	89 ± 10	95 ± 3
Albumin	3 ± 0.2	1.63* ± 0.1	1.41* ± 0.3	1.63* ± 0.6	1.64* ± 0.1	1.80* ± 0.2	2.2* ± 0.1
Ammonia Urea (N)	25.1 ± 7	12.2* ± 1.5	10.1* ± 1.5	5.89* ± 3.6			
AAA (umole/100 ml)	18.3 ± 1.2			45 ± 1			20 ± 3
BCAA (umole/100 mol)	46.3 ± 1.7			64.7 ± 0.7			50 ± 1
BCAA/AAA	2.55 ± 0.21			0.99* ± 0.09			2.5 ± 0.12

The values are expressed as mean ± SD.

*$P < .05$ when the values are compared with the basal levels.

†$P < .05$ when the values of nonsurviving dogs are compared with the values of the surviving dogs.

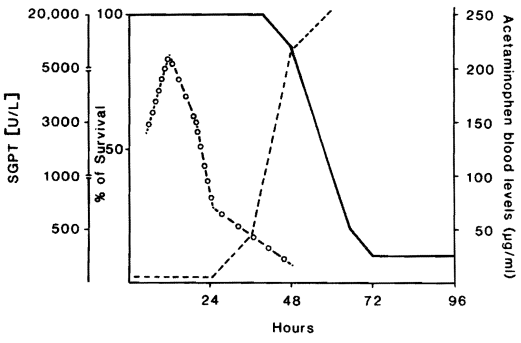

Fig 1. Survival rate, blood acetaminophen levels, and serum glutamic-pyruvic transaminase (SGPT) levels *v* time in treated dogs. The survival rate [—] is expressed as %, the SGPT levels [---] expressed as U/L, and the blood acetaminophen level [O—O—] as µg/mL.

necrosis with reticulum collapse and occasional central-central bridging necrosis. The degree of necrosis varied from involvement of one-third of the lobule in the least severely affected areas to almost panlobular necrosis with only a thin rim of viable periportal hepatocytes remaining in the more severely affected areas.

No change in transaminase levels, alteration in liver or kidney histology, or deaths occurred in control, DMSO treated, animals.

Table 1 lists the various biochemical determinations obtained in the animals. Nonsurviving animals demonstrated, in addition to a rapid increase of transaminase levels, a significant decrease in cholesterol, albumin, and

urea nitrogen, which occurred during the first 48 hours of acetaminophen intoxication. The plasma ammonia level increased rapidly during the same time period. In the surviving animals, the degree of deterioration of all these same parameters was in the same direction, but to a lesser degree. The maximum levels of transaminases in these animals was only 1,100 U/mL and the levels of cholesterol, albumin, urea, and ammonia did not decline to the levels observed in the nonsurviving animals. Table 1 also lists the determinations of the BCAA, AAA, and BCAA/AAA ratio. A rapid increase in AAA was observed in all animals, but a more pronounced elevation was observed in the nonsurviving dogs.

In conclusion, our results report for the first time a model of acute hepatic failure in a large animal that can be easily reproduced in any laboratory. This model satisfies all of the criteria for a suitable animal model of acute hepatic failure.[4]

The use of this model in liver transplantation will be useful to better define the clinical indications for orthotopic liver transplantation, and to gain further insight into the pathophysiology of this acute and deadly process.

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