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Effect of Ranitidine on Acetaminophen-Induced Hepatotoxicity in Dogs

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The effect of ranitidine administration upon the hepatotoxic effect produced by a multidose acetaminophen administration regimen was examined. Seventy-two dogs received three subcutaneous injections of acetaminophen (750, 200, 200 mg/kg body wt) in DMSO (600 mg/ml) at time zero, 9 hr later, and 24 hr after the first dose. Ten control animals (group I) were not given ranitidine, the remaining 62 dogs received an intramuscular injection of ranitidine 30 min before each acetaminophen dose. Three different doses of ranitidine were used (mg/kg body wt): 50 mg, group II (33 dogs); 75 mg, group III (14 dogs); 120 mg, group IV (15 dogs). Ranitidine reduced the expected acetaminophen-induced hepatoxicity in a dose–response manner. Moreover, a significant correlation was found between the ranitidine dose and the survival rate, as evidenced by transaminase levels in the serum and histology of the liver. This model of fulminant hepatic failure induced by acetaminophen and its modulation with ranitidine provides clinical investigators with a research tool that will be useful in the future investigation of putative medical and surgical therapies being investigated for use in the clinical management of fulminant hepatic failure. Because of the size of the animal used in this model, frequent and serial analyses of blood and liver were available for study to determine the effect of therapy within a given animal as opposed to within groups of animals.

KEY WORDS: hepatotoxicity; acetaminophen; ranitidine.

Recently, a large-animal model of acute hepatic failure using acetaminophen was described (1). The two major innovations of this model include the use of DMSO (which does not affect transaminase levels or liver and kidney histology) as the vehicle for the administered acetaminophen and a serial dosage schedule which maintains a hepatotoxic level of acetaminophen in the animals' blood for several hours, thereby producing a more severe and consistent hepatic necrosis with a mortality rate of 90% (1). All other models of fulminant hepatic failure, particularly those using rats (2), rabbits (3) have been difficult to standardize, are not reproducible, and do not allow for serial observations to be made in animals that may be recovering. The use of large and single doses of ranitidine prior to the administration of acetaminophen in the commonly used rat model of fulmi-

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nant hepatic failure (7) appears to reduce the subsequent mortality and improve the survival rate of the animals (8). This effect depends on the ability of this H_2-receptor antagonist to bind cytochrome P-450 (9) and inhibit microsomal hepatic mixed-function oxidase activity. Although cimetidine is known to be the most powerful agent among H_2-receptor antagonists with respect to its effect on cytochrome P-450 (10), we used ranitidine because it does not affect hepatic regeneration, as previously shown by us and by others (11–13).

In this paper, the effect of different doses of ranitidine on acetaminophen-induced hepatotoxicity in dogs was evaluated to develop a model for acute hepatic failure with a predetermined mortality rate.

MATERIALS AND METHODS

Chemicals. Acetaminophen and DMSO were purchased from Sigma Chemical Co., St. Louis, Missouri; pentothal was purchased from Abbott Laboratories, North Chicago, Illinois; ranitidine was obtained from Glaxo, Inc., Research Triangle Park, North Carolina; xylocaine was purchased from Astra Pharmaceutical Products, Ind., Worchester, Massachusetts; and formaldehyde was obtained from Fisher Scientific, Pittsburgh, Pennsylvania.

Animals. Seventy-two male beagles were purchased from Russel B. Hutton Farms, St. Thomas, Pennsylvania. The dogs were housed in a large animal care facility, kept at a constant temperature of 20 ± 1° C and with a 0600- to 1800-hr light–dark schedule. Dogs were quarantined and allowed to acclimate to their surroundings for a minimum of one week before use. All dogs were given dry dog food and water *ad libitum*. Prior to study, their body weights ranged from 9–14 kg.

Study Design. The animals were randomly divided into four different groups.

Group I consisted of 10 dogs that 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 body wt) was given at noon; the second injection (200 mg/kg body wt) was given 9 hr later; the third dose (200 mg/kg body wt) was given 24 hr after the initial dose.

Groups II–IV consisted of 62 dogs that were administered the acetaminophen using the same schedule as the dogs in group I but, in addition, were given ranitidine intramuscularly 30 min before each dose of acetaminophen at one of the following dosages: 50 mg/kg body wt, group II (33 dogs); 75 mg/kg body wt, group III (14 dogs); 120 mg/kg body wt, group IV (15 dogs). All animals were provided with intravenous glucose in amounts necessary to maintain their blood sugar within normal limits as defined as ±15% of the basal.

Biochemical Determinations. In all dogs (groups I–IV), serum glutamic-pyruvic transaminase levels (SGPT-ALT) were determined before and at scheduled times after acetaminophen administration using standard laboratory methods. Acetaminophen blood levels were determined in groups I and IV animals using the method described by Routh et al (14).

Histology. All animals that died underwent a full necropsy, which included a histologic evaluation of both the liver and kidneys.

Three dogs from group IV were sacrificed at 72 hr for histological evaluation because experience with the model had shown the histologic injury to be maximal at this time point and the probability of a long-term survival in an animal surviving to this point in time is greater than 90%.

The histology assessment of the degree of tissue necrosis present was performed using a semiquantitative scale. This scale was based on two observations: the extent of necrosis within individual lobules, which was classified as (1) mild = less than ⅓ of the lobule, (2) moderate ⅓–⅔ of the lobule, and (3) more than ⅔ of the lobules being involved; and the number of hepatic lobules affected with (1) being less than ⅓, (2) ⅓–⅔, and (3) greater than ⅔ of the lobules being affected. A score for each variable assessed was obtained and a mean of the two variables was used as the component score for the biopsy. These scores could range from 0–6 with no injury in any lobule to massive necrosis in greater than ⅔ of all lobules. Tissues were fixed in 10% neutral buffered formalin, sectioned at 5 μm and stained with hematoxylin and eosin.

Statistical Analysis. The statistical analysis for the biochemical determinations was performed using one-way analysis of variance (SPCC/PC statistical software, SPSS, Inc., Chicago, Illinois). The unpaired Student's *t* test was used for the analysis of the survival rates in groups I–IV. The relationship between long-term survival and the dose of ranitidine administered was examined using probit analysis (15). A weighted regression equation was used with an adjustment for natural mortality. A value of $P < 0.05$ was considered to be significant.

RESULTS

Animal Survival. The survival rate and time course of the experiment for the dogs in groups I–IV are summarized in Figure 1. No deaths occurred within the first 24 hr. A 10% mortality was seen in all groups except group IV within the next 24 hr. From 48 through 72 hr, a progressive increase in mortality was observed in all four groups. A mortality of 90% at 72 hr was achieved in the animals in group I. In group II, animals given ranitidine at a dose of 50 mg/kg body wt, a small (from 90% to 82%) but insignificant reduction in the mortality rate at 72 hr was observed. In group III, animals given ranitidine at a dose of 75 mg/kg body wt, a substantial reduction in the mortality rate (50%) at 72 hr was observed ($P < 0.05$). In group IV, animals given ranitidine at a dose of 120 mg/kg

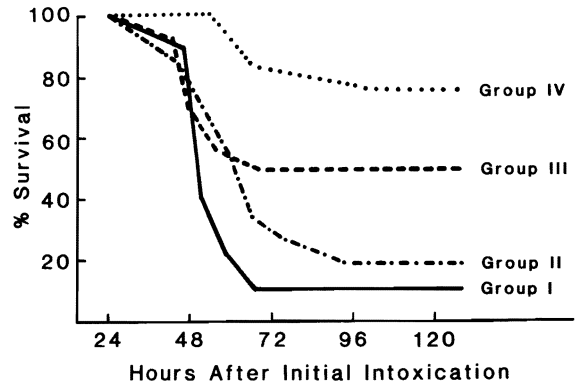

Fig 1. Survival rate versus time in group I–IV dogs. All the animals received the same acetaminophen treatment. In addition they received a different intramuscular dose of ranitidine 30 min before each acetaminophen administration (mg/kg). Group I (control), 0 mg; group II, 50 mg; group III, 75 mg; group IV, 120 mg.

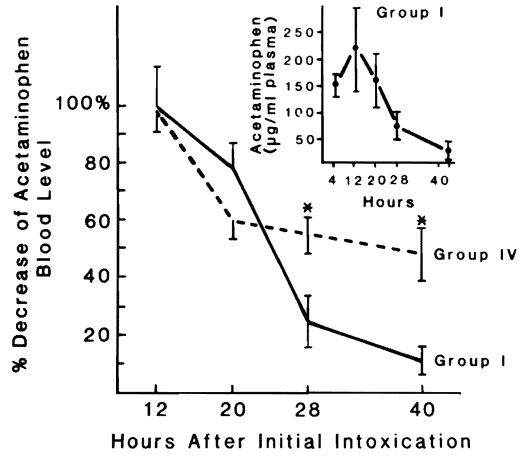

Fig 2. Acetaminophen plasmatic levels of dogs in groups I and IV. Each value (mean ± SD) represents the value obtained in four dogs at each time. *Significantly different from the value obtained in the control animals (group I) at the same time ($P < 0.05$). Inset: acetaminophen plasma levels in the animals of group I after initial injection.

body wt, the mortality rate was reduced to only 25% at 72 hr ($P < 0.05$). Thus, a statistically significant dose–response in terms of survival was observed with increasing doses of ranitidine from 50 to 120 mg/kg body wt.

Biochemical Parameters. Table 1 lists the serum ALT (SGPT) values in the animals at each time point. No difference for ALT values between groups of non surviving dogs was evident. Similarly, no difference between groups of surviving animals for transaminase values was seen. However, a significant difference was observed between the transaminase values of surviving and nonsurviving animals across time. Surviving animals appeared to have both a reduced and delayed release of transaminase into serum than did the nonsurviving animals.

Figure 2 demonstrates the plasma levels of acetaminophen observed in the animals of groups I and IV across time. A significant difference ($P < 0.05$) in acetaminophen plasma levels after 28 hr was seen between the animals in groups I and IV and persisted through 40 hr after the initial dosing of the animals. The actual acetaminophen levels present in the blood of dogs in group I are shown in the inset.

Histopathology. The liver tissue obtained from the nonsurviving animals of each group demonstrated severe centrilobular necrosis involving 100% of the hepatic lobules, reticulin collapse (Figure 3A and B), and occasional areas of central–central bridging necrosis. The extent of the necrosis within individual lobules varied from a low of 40% in the least severely affected animals to essentially 100% necro-

TABLE 1. SERUM GLUTAMIC-PYRUVIC TRANSAMINASES (ALT-SGPT) IN NONSURVIVING AND SURVIVING DOGS FROM GROUPS I–IV AT DIFFERENT TIMES AFTER INTOXICATION.*

	ALT-SGPT (units/liter)					
	0.0 hr	*24 hr*	*48 hr*	*72 hr*	*96 hr*	*120 hr*
Nonsurviving						
Group I	40 ± 14	75 ± 50	2,631[ab] ± 2,696	19,980[ab] ± 4,200		
Group II	38 ± 17	69 ± 43	3,507[ab] ± 1,493	18,601[ab] ± 5,426	17,500[ab] ± 6,100	
Group III	29 ± 10	81 ± 46	3,001[ab] ± 703	12,970[ab] ± 4,521		
Group IV	42 ± 15	47 ± 21	3,204[ab] ± 1,776	12,401[ab] ± 3,203		13,601[ab] ± 2,000
Surviving						
Group I	31 ± 14	64 ± 31	580[a] ± 278	1,706[a] ± 401	1,800[a] ± 503	1,024[a] ± 426
Group II	37 ± 15	61 ± 30	711[a] ± 200	2,489[a] ± 1,900	2,907[a] ± 821	7,709[a] ± 793
Group III	38 ± 17	65 ± 30	810[a] ± 600	3,279[a] ± 3,250	3,126[a] ± 2,613	2,017[a] ± 1,975
Group IV	40 ± 15	59 ± 33	799[a] ± 601	2,900[a] ± 2,780	3,214[a] ± 2,789	7,905[a] ± 9,582

*The values are expressed as mean ± SD. [a], $P < 0.05$ when the values are compared to the basal levels. [b], $P < 0.05$ when the values of nonsurviving dogs are compared with the value of the surviving dogs of the same group.

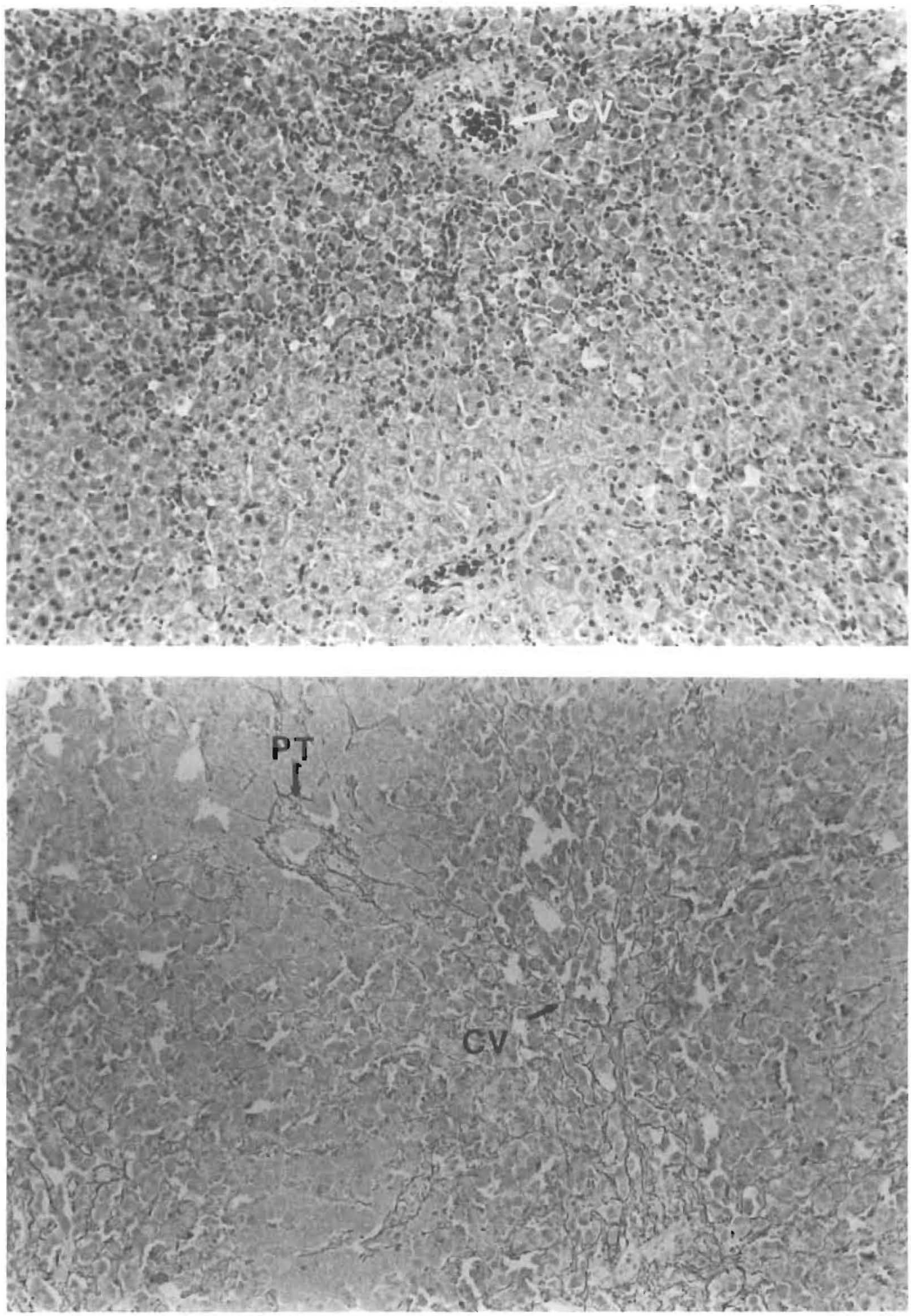

Fig 3. Characteristic histopathologic appearance of liver in dogs from group I. (A) Note severe centrilobular necrosis and hemorrhage with some preservation of periportal hepatocytes (portal tract at bottom center) (H & E, 200×). (B) The damage resulted in collapse of the perivenular reticulin architecture with preservation at periportal areas (Reticulin stain, 200×). PT = portal tract; CV = central vein.

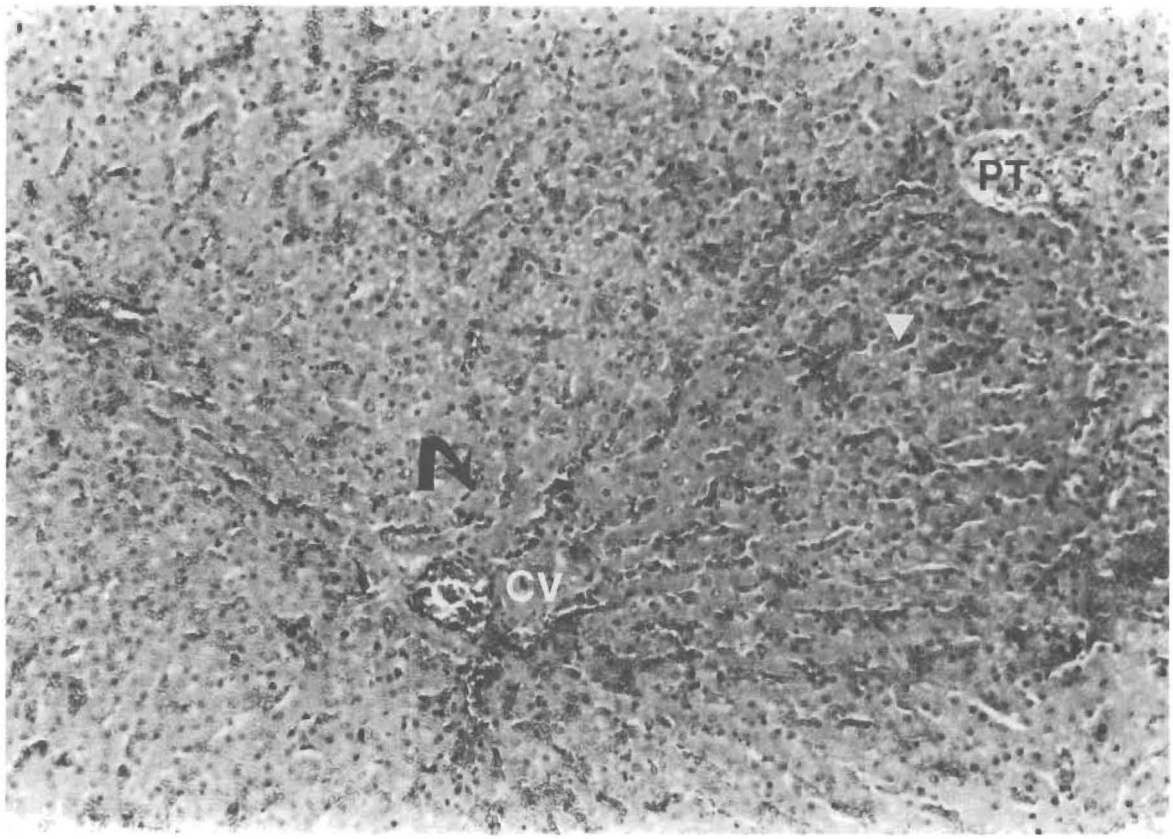

Fig 4. Histopathologic appearance of liver in dogs from group IV (H & E, 400×, PT = portal tract, CV = central vein). Note that there is centrilobular congestion, but centrilobular hepatocytes are intact and viable (arrow). Microvesicular stenosis was also detected in hepatocytes (arrowhead).

sis with only a thin rim of viable periportal hepatocytes remaining in the more severely affected animals. The hepatocellular necrosis in each case studied was associated with centrilobular congestion. Individual necrotic cells demonstrated cytoaggregation, eosinophilia, nuclear pyknosis, karyorrhexsis, and karyolysis. The few residual viable periportal hepatocytes demonstrated cellular swelling and microvacuolization.

The histopathology of the three randomly selected dogs in group IV that were sacrificed for study having a predicted 90% change of survival demonstrated a zonal necrosis involving no more than 70% of the lobules (Figure 4). In addition, the degree of necrosis within individual lobules varied from a low of 10% to a high of 70%. The cells in the necrotic areas demonstrated cytological alterations similar to those described in the liver specimens obtained from the nonsurviving dogs. No histologic alterations were evident in the kidneys obtained from either the surviving or nonsurviving dogs in any of the groups studied.

Relationship between Survival and Ranitidine Dosage. Figure 5 illustrates the relationship between survival at 72 hr and thereafter and the ranitidine dosage used. The curve obtained fits the following function: probit = $-2.48 + 3.94$ ($\log_{10}$ dose). This function predicts the expected mortality at different dosages of ranitidine between 50 and 150 mg/kg

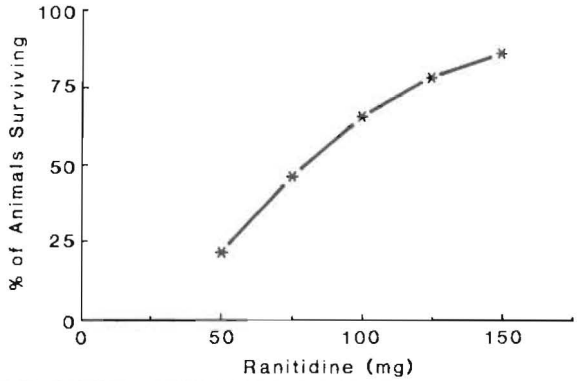

Fig 5. Relationship between final percentage of animal survival and the ranitidine dosage used in each group of dogs studied.

TABLE 2. CONFIDENCE INTERVALS FOR ESTIMATED PROBITS OF SURVIVAL RATE AT DIFFERENT DOSES OF RANITIDINE

Ranitidine dose (mg)	*Estimated survival %*	*95% of confidence interval for % survival*
50	21.8	12.6 – 34.0
75	46.6	36.5 – 57.0
100	65.8	51.3 – 78.4
125	78.6	60.6 – 90.5
150	86.5	67.4 – 96.0

body wt. Table 2 lists the survival at each dosage of ranitidine used using the probit analysis. Note that except for the higher dose of ranitidine (>100 mg/kg body wt), little overlap exists between the figures. Moreover, despite some overlap, a group enhancement of survival occurred with each incremented dose of ranitidine used.

DISCUSSION

A large animal model (1, 8) of acetaminophen-induced hepatic failure using a protocol of multidose acetaminophen administration is described. This model is highly reproducible and provides clinical investigators with a much needed large-animal model of fulminant hepatic failure that will allow within-animal assessments of liver injury using serial blood sampling and liver biopsy methods of assessment.

The results reported demonstrate that it is possible to ameliorate acetaminophen-induced hepatotoxicity in this large animal model in a dose-dependent manner with the use of ranitidine administered at doses ranging from 50 to 120 mg/kg body wt. Using a probit analysis, it is possible to predict the expected mortality for each dose of ranitidine. This should provide future investigators a predictable model of fulminant hepatic failure for any given experimental purpose that might be investigated. This model should be particularly useful for investigators planning to evaluate the effects of putative therapies for fulminant hepatic failure in that it provides animals with predictably different mortality rates for study.

The protective effect of ranitidine observed in this animal model is due to its capacity to bind cytochrome P-450 and prevent the oxidation of acetaminophen to its toxic metabolites (16–18). Ranitidine was chosen for these experiments instead of cimetidine, which is a more powerful inhibitor of P-450 enzyme systems, in an effort to avoid the "hormonal" antiandrogenic influence of cimetidine upon the expected hepatic regeneration (11–13). The data presented in Figure 2 are consistent with such a hypothesis, but do not prove it. The amount of acetaminophen administered to the animals in group I was sufficient to induce a mortality rate of 90%.

Ranitidine, particularly at very high doses, is known to bind to P-450 and inhibits the metabolism of acetaminophen. Such an effect was observed as ranitidine reduced both the rate of acetaminophen disappearance from blood (Figure 2) and the mortality experienced (Figure 1).

It is of some interest that no correlation between the degree of hepatic injury and the dose of ranitidine administered to surviving animals was evident. The surviving animals all appeared to have a delayed peak in transaminase levels, probably reflecting a delayed production of putative acetaminophen toxic metabolites. The histopathologic studies performed in the dogs from group IV, which were sacrificed, clearly demonstrates the problems that arise in attempting to prognosticate using small tissue samples. Despite the low mortality rate of this particular group, 60 ± 20% of the hepatic lobules were involved in the necrotic process and the degree of necrosis within individual lobules varied widely (42 ± 20%).

On the basis of the data presented here, the following four major conclusions can be drawn: (1) a multidose acetaminophen injection protocol used in conjunction with ranitidine administration is a reproducible method for inducing acute hepatic failure in dogs with a predetermined mortality rate, depending upon the dose of ranitidine used; (2) with increasing doses of ranitidine (0–120 mg/kg body wt), the mortality can be reduced predictably from 90% to 25%; (3) probit analysis allows one to predict the expected mortality in this model for any dose of ranitidine used by the investigation; and (4) this model of acute hepatic failure should provide clinical investigators with a malleable tool to test the effect of putative therapies for fulminant hepatic failure.

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