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Modification of the Hepatotoxicity of D-Galactosamine in the Rat by Cycloheximide (39248)

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Administration of the aminosugar, D-galactosamine, results in profound histological and biochemical evidence of hepatocellular injury in the rat (1, 2). Diffuse necrosis of hepatocytes is accompanied by an inflammatory cell infiltrate consisting of polymorphonuclear leukocytes, eosinophils, lymphocytes, and macrophages. In addition, fatty liver due to an accumulation of triglyceride is prominent in treated animals (2, 3). Shortly after the administration of hepatotoxic doses of D-galactosamine alterations of the circular dichroism spectrum and a reduction of the 5′ nucleotidase activity of hepatocyte plasma membranes have been observed, and total liver calcium ion concentration rises (4). Impairment of macromolecular biosynthesis has been demonstrated (3, 5–7), and it has been suggested that the accumulation of UDP hexosamines and a deficiency of hepatic uridine nucleotides and UDP hexoses are responsible for the hepatotoxic action of D-galactosamine, which can be reversed by the administration of uridine. The precise mechanism of D-galactosamine-induced hepatocyte necrosis and its relationship to injury of the plasma membrane, organelles, or impaired biosynthetic pathways remains, however, poorly understood.

In the present report we provide evidence that pretreatment or simultaneous administration of cycloheximide, a potent inhibitor of hepatic protein synthesis (8), modifies the hepatotoxicity produced by D-galactosamine. These data support the notion that protein synthesis may play an important role in the pathogenesis of hepatocellular injury.

Material and methods. Female rats of the CD strain, weighing 160–200 g, were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. D-Galactosamine HCl and cycloheximide were obtained from Sigma Chemical, St. Louis, Missouri. Animals were allocated to five experimental groups. Animals in Group I were given D-galactosamine dissolved in isotonic saline, intraperitoneally (ip) in a single dose of 375 mg/kg of body weight. Group II animals received only cycloheximide, in isotonic saline, by injection in a dose of 1.5 mg/kg. This dose has been shown to inhibit protein synthesis *in vivo* in rat liver cells by greater than 90% (9). RNA synthesis is not impaired. Furthermore this dose of cycloheximide has been shown to have no important effect on plama free fatty acids or hepatic triglyceride levels and produces only minimal ultrastructural change in the hepatocyte (10).

Groups III, IV, and V received both D-galactosamine and cycloheximide. In Group III cycloheximide was given 4 hr after D-galactosamine, whereas in Group V cycloheximide was given 4 hr before D-galactosamine. Animals in Group IV received both agents simultaneously. Following administration of these agents animals were fasted but allowed *ad libitum* access to water. Twenty-four hours after D-galactosamine administration in Groups I, III–V, and 24 hr after cycloheximide administration in Group II, animals were sacrificed by exsanguination. A subgroup of animals in Groups I and V were sacrificed 48 hr after injection of D-galactosamine.

Serum, obtained at the time of sacrifice, was assayed in duplicate for glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) by spectrophotometric methods (2). Enzyme activity was expressed as units per milliliter per minute. Samples of each liver were fixed in buffered 10% formalin. The extent of hepatocellular necrosis was assessed by light microscopic examination of coded hematoxylin and eosin-stained paraffin sections. Necrosis was graded on a scale of 0 to 4+ and mean

scores were determined after decoding.

Hepatic triglycerides were extracted from other portions of each liver and, after thin-layer chromatography, were quantitated by standard methods (2). Hepatic triglyceride values were expressed as milligrams of triglyceride per gram of wet weight of liver.

Statistical analyses were made with Student's *t* test and chi-square.

Results. Serum enzymes after D-galactosamine and cycloheximide. As indicated in Table I, SGOT and SGPT were strikingly elevated 24 hr after injection of D-galactosamine in Group I animals, when compared to Group II animals receiving cycloheximide alone. The values obtained in Group II animals were in fact similar to those obtained by us previously in saline-injected animals (2). SGOT and SGPT in Group III (cycloheximide 4 hr after D-galactosamine) were not statistically significantly different from D-galactosamine-treated Group I animals. In contrast, animals in Groups IV and V (cycloheximide simultaneously or 4 hr before D-galactosamine) had significantly lower serum transaminase levels.

Forty-eight hours after D-galactosamine, serum transaminases, as shown in Table II, were significantly lower in survivors of Group V in comparison with survivors of Group I. The fatality rate in Group I, at 48 hr, was 69% (9/13), while in Group V fatality was 14% (2/14). These fatality rates were significantly different (chi-squared = 8.4; $P < 0.01$).

Histologic grading of hepatocellular necrosis. Twenty-four hours after D-galactosamine, an average necrosis score of 3.7+ was found in Group I animals. Group II animals (cycloheximide alone) showed no necrosis at 24 hr. Necrosis scores in Groups IV and V were 2.3+ and 1.2+, respectively. Animals in Group III (cycloheximide after D-galactosamine) had an average score of 3.3+. Necrosis at 48 hr in survivors was scored as 2.8+ in Group I, in contrast to a mean score of 1.5+ in Group V. Thus histologic grading of the severity of necrosis appeared to correlate with transaminase levels obtained in these animals.

Hepatic triglycerides. As indicated in Table III, hepatic triglycerides were elevated in D-galactosamine-treated animals, as compared to animals receiving cycloheximide alone. Treatment with cycloheximide in Groups III, IV, and V reduced the triglyceride accumulation associated with D-galactosamine, but a statistically significant reduction was observed only in Group IV animals (D-galactosamine and cycloheximide

TABLE I. SERUM TRANSAMINASES IN RATS 24 HR AFTER D-GALACTOSAMINE OR CYCLOHEXIMIDE.

Group	D-galactosamine (375 mg/kg)	Cycloheximide (1.5 mg/kg)	SGOT* Mean ± SE (number of animals)	SGPT* Mean ± SE (number of animals)
I	+	0	11,063 ± 1389 (4)	2934 ± 190 (4)
II	0	+	113 ± 19 (5)	21 ± 6 (5)
III	+	+ (4 hr after D-galactosamine)	7190 ± 2237 (5)	2068 ± 628 (5)
IV	+	+ (Together)	1192 ± 354 (11)	371 ± 151 (11)
V	+	+ (4 hr before D-galactosamine)	334 ± 68 (11)	108 ± 27 (11)

* Values expressed as units per milliliter per minute. Group I vs Group II: $P < 0.001$ for SGOT and SGPT. Group I vs Group III: NS. Group I vs Groups IV and V: $P < 0.001$ for SGOT and SGPT.

TABLE II. MORTALITY 48 HR AFTER D-GALACTOSAMINE AND SERUM TRANSAMINASES IN SURVIVING RATS IN GROUPS I AND V.

	Group	Fatalities/Total number of animals	SGOT[a] (mean ± SE)	SGPT[a] (mean ± SE)
I	(D-galactosamine alone)	9/13[c]	4863 ± 1473[b]	1519 ± 372[d]
V	(Cycloheximide before D-galactosamine)	2/14	2185 ± 409	628 ± 96

[a] Values expressed as units per milliliter per minute.
[b] SGOT (survivors of Group I vs V): $P < 0.05$.
[c] Fatality (Group I vs V): $P < 0.01$.
[d] SGPT (survivors of Group I vs V): $P < 0.01$.

TABLE III. HEPATIC TRIGLYCERIDES 24 AND 48 HR AFTER D-GALACTOSAMINE.

Group	D-galactosamine (375 mg/kg)	Cycloheximide (1.5 mg/kg)	Hepatic Triglycerides (mg/g)	
			24 hr[a] Mean ± SE (number of animals)	48 hr[a] Mean ± SE (number of animals)
I	+	0	21.0 ± 3.6 (4)	26.8 ± 5.0 (4)
II	0	+	7.0 ± 3.1 (5)	–
III	+	+ (4 hr after D-galactosamine)	11.7 ± 3.2 (5)	–
IV	+	+ (Together)	8.5 ± 2.5 (11)	–
V	+	+ (4 hr before D-galactosamine)	12.5 ± 2.6 (11)	14.3 ± 2.1 (12)

[a] Group I vs II: $P < 0.02$. Group I vs IV: $P < 0.02$.
[b] Group I vs V: $P < 0.02$.

simultaneously). Hepatic triglycerides were significantly reduced at 48 hr in surviving animals treated with cycloheximide before D-galactosamine when compared to those levels found in rats treated with D-galactosamine alone.

Discussion. Decker and Keppler (11) have suggested that depression of uridine nucleotide dependent production of macromolecules leads to organelle injury and subsequent necrosis of liver cells in D-galactosamine-treated animals. However, a dissociation of inhibition of macromolecular synthesis and cell necrosis has been demonstrated recently with D-galactosamine. Inhibition of RNA and protein synthesis by approximately 90% has been produced by small doses of D-galactosamine (200 mg/kg) which do not cause hepatocyte necrosis (6). Larger doses of D-galactosamine (400 mg/kg) produce a similar degree but more persistent inhibition of macromolecular synthesis, and result in extensive hepatocyte necrosis (6). These observations suggest that D-galactosamine-induced inhibition of RNA and protein synthesis is not related per se to the liver cell necrosis associated with D-galactosamine.

El-Mofty *et al.* (4) have reported that D-galactosamine induces early alterations in hepatocyte plasma membranes and calcium permeability, and suggested that these changes are causally related to subsequent cell necrosis. A disturbance of the carbohydrate components of the plasma membrane was postulated, but the specific biochemical lesion responsible and its relationship to cell necrosis remains speculative. It is possible that alterations in the protein composition of the plasma membrane may underlie the observed plasma membrane injury.

Recent studies, employing other models, have suggested that necrosis may be due to the formation and/or accumulation of new proteins rather than the inhibition of macromolecular and protein biosynthesis (12). The results of the present study support this notion since administration of cycloheximide, an agent which blocks the elongation of nascent peptides and the initiation of new peptide chains (13), appears to ameliorate D-galactosamine hepatotoxicity, as reflected by hepatocyte necrosis and hepatic triglyceride accumulation. This protective effect was observed when cycloheximide was given 4 hr prior to or simultaneously with D-galactosamine, but could not be demonstrated if given 4 hr after D-galactosamine. Rumpelt *et al.* (14) found, by light and electron microscopic studies, that pretreatment of rats with cycloheximide, in an amount 3 to 10 times greater than that used by us, resulted in a diminution or complete absence of the focal hepatocyte cytoplasmic degeneration observed in animals receiving D-galactosamine alone. No biochemical evidence of a cycloheximide effect on D-galactosamine-induced hepatic necrosis was presented.

In the rat, cycloheximide has been shown to prevent or reduce puromycin-induced pancreatic acinar cell damage (15), intestinal epithelial cell necrosis produced by x-irradiation, nitrogen mustard, or cytosine arabinoside (16), and hepatocyte necrosis induced by diethylnitrosamine, 2-acetylaminofluorene, 3′-methyl-4-dimethylaminoazobenzene, and carbon tetrachloride (17). Thus, modification of cell necrosis by cycloheximide is limited neither to the liver nor to specific toxic agents. Whether cycloheximide exerts its modifying action as a consequence of its inhibition of protein synthesis

or through now unknown effects on cell metabolism or upon the plasma membrane is uncertain. It has been shown, for example, that cycloheximide can inhibit gluconeogenesis and mitochondrial energy transfer in rat liver and lipogenesis in rat adipose tissue (18). Studies with other inhibitors of protein synthesis in D-galactosamine-induced hepatic injury may shed more light on the mechanism of cycloheximide's action in this model.

Summary. The effect of cycloheximide (1.5 mg/kg), a potent inhibitor of protein biosynthesis, on D-galactosamine (375 mg/kg)-induced hepatic necrosis and hepatic triglyceride accumulation was studied in rats. Serum transaminase levels, 24 hr after D-galactosamine administration, were significantly reduced in animals treated simultaneously or 4 hr before D-galactosamine with cycloheximide, when compared to animals given D-galactosamine alone. Transaminase levels in rats given cycloheximide 4 hr after D-galactosamine were not reduced. Histological grading of hepatocyte necrosis showed a similar pattern of protection in the pretreated and simultaneously treated groups. Hepatic triglycerides were significantly reduced only in the latter group.

Fatality 48 hr after D-galactosamine administration was significantly less common in rats pretreated with cycloheximide when compared to rats given D-galactosamine without cycloheximide, and surviving animals in the cycloheximide pretreated group had a lower serum transaminase level, a lower necrosis score, and a reduced hepatic triglyceride level.

These data are consistent with the concept that protein synthesis is important in the pathogenesis of D-galactosamine-induced hepatotoxicity.

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