PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 903–907 (1975)

Potentiation of the Hepatotoxic Responses to Chemicals in Alloxan-Diabetic Rats[1, 2] (38924)

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Numerous clinical and experimental data have shown that a variety of chemicals occasionally will modify the toxic action of chemicals upon certain organs. By comparison, however, fewer studies exist in which the effects of experimentally induced disease states on the toxicity of chemicals have been examined.

In a previous study (1) we reported that the centrilobular necrosis produced by carbon tetrachloride is markedly enhanced in rats during diabetic states induced by either alloxan or streptozotocin. Therefore we sought to determine if the actions of a variety of other hepatotoxic agents could also be potentiated in alloxan-diabetic rats.

We began by testing the effects of alloxan pretreatment on the liver damage produced by chloroform, 1,1,2-trichloroethane, trichloroethylene, and 1,1,1-trichloroethane, each having a lower hepatotoxic potential than that of carbon tetrachloride (2, 3).

In other experiments, we examined the effects of alloxan on the hepatotoxic responses to chemical agents whose effects differ from those resulting from the aforementioned chlorinated hydrocarbons. The hepatotoxins comprising this second group were galactosamine (focal necrosis) (4), beryllium (midzonal necrosis) (5), and alpha-naphthylisothiocyanate (cholestasis) (6, 7).

Methods and Materials. Male Sprague–Dawley rats (Canadian Breeding Farm and Laboratories, St-Constant, Quebec) weighing 230–320 g were given single intravenous injections of alloxan monohydrate (Eastman Chemical Co.) at a dosage of 60 mg/kg. The drug solution was prepared in saline and administered in a volume of 1.0 ml/kg. Rats not pretreated with alloxan were given only saline by the intravenous route.

Three days after alloxan pretreatment, the animals were either challenged with single doses of a given hepatotoxic agent, or given the vehicle only. The rats were then sacrificed 24 hr later to assess the extent of liver injury.

Chloroform, 1,1,2-trichloroethane, trichloroethylene, and 1,1,1-trichloroethane were injected intraperitoneally at dosages of 0.5, 0.05, 1.0, and 1.0 ml/kg, respectively. Each of the compounds was administered at a dosage which produced little or no elevation of serum glutamic pyruvic transaminase (SGPT) when given to nonalloxan-pretreated rats under the conditions described above. All halogenated hydrocarbons were solubilized in corn oil and given in an injection volume of 4.0 ml/kg.

Alpha-naphthylisothiocyanate (ANIT) (Eastman Chemical Co.) was dissolved in corn oil (33.3 mg/ml) and given orally at a dosage of 300 mg/kg. Galactosamine HCl (Sigma Chemical Co.) was solubilized in saline (100 mg/ml) and given intravenously (200 mg/kg). $BeSO_4 \cdot 4H_2O$ (Fisher Scientific) was dissolved in saline and injected intravenously at a dosage of 0.15 mg Be/kg.

Biochemical assessment of liver injury was based upon one or two of the following measurements: SGPT activity (8), hepatic triglyceride concentration (9), plasma isocitric dehydrogenase (ICDH) activity (5), and total bilirubin concentration in plasma (10). These parameters have been found, in appropriate studies, to be good indicators of liver injury for the compounds under study.

Plasma glucose concentrations, determined by the orthotoluidine method (glucose reagent kit, Harleco, Co.), were measured at the time of sacrifice to verify the hyperglycemic state induced by alloxan. The

[1] From the Medical Research Council Group in Drug Toxicology.

[2] This investigation supported by the Medical Research Council of Canada.

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range of plasma glucose concentrations for all treatment groups given alloxan was 250–2100 mg/100 ml.

In one series of experiments, rats were given alloxan as described above. Twenty-four hours later, blood samples were taken from the tail veins of these animals to ascertain that plasma glucose concentrations were above 200 mg/100 ml. Half of the animals in this group were then given single daily subcutaneous injections of protamine zinc insulin at a dosage of 10 units/kg/day for 3 days, while the other half received saline injections only. Three days after alloxan pretreatment, all rats were challenged with chloroform (0.5 ml/kg, ip) and sacrificed 24 hr later.

Statistically significant differences among treatment groups were determined by the Mann–Whitney U Test (11) at $P \leqslant 0.05$ (one-tail test), except for the ICDH activities where Student's t test (12) was employed ($P < 0.05$).

Results. The effects of alloxan pretreatment on the liver injury induced by chloroform or 1,1,2-trichloroethane are shown in Table I. Alloxan pretreatment alone produced no significant changes in SGPT activity or in hepatic triglyceride concentrations when compared to control rats (non-alloxan pretreated) given the corn oil vehicle.

The challenging dose of chloroform or 1,1,2-trichloroethane, when given to non-alloxan-pretreated rats resulted in SGPT activities which were similar to that of unchallenged controls. However, the same challenging dose of each haloalkane, when given to alloxan-diabetic rats produced SGPT values which were approximately 63-fold higher for chloroform, and 17-fold higher for 1,1,2-trichloroethane.

Hepatic triglyceride concentrations were elevated by about fivefold in alloxan-pretreated rats challenged with 1,1,2-trichloroethane. Alloxan, however, did not alter hepatic triglyceride concentrations in rats challenged with chloroform.

The marked potentiation of SGPT activity with chloroform in alloxan-diabetic rats prompted us to determine if insulin treatment during the diabetic state could protect against the chloroform-induced elevation of activity of this serum enzyme (Table II). A comparison of the changes in plasma glucose concentrations when measured 24 hr after alloxan (when insulin treatment began), and again at the time of sacrifice, show that the hyperglycemic response to alloxan was lessened by the dosage of insulin employed. Under these conditions, insulin treatment markedly lowered the chloroform-induced elevation of SGPT in

TABLE I. EFFECTS OF ALLOXAN DIABETES ON LIVER INJURY INDUCED BY HALOGENATED HYDROCARBONS IN MALE RATS[a]

Treatment	SGPT (units/ml)	Hepatic triglyceride concentration (mg/g)
Control + corn oil	36 ± 3	6.1 ± 0.5
Alloxan + corn oil	56 ± 7	9.0 ± 3.2
Control + chloroform	48 ± 3	10.1 ± 1.6
Alloxan + chloroform	3000 ± 1120[b]	12.4 ± 2.9
Control + 1,1,2-trichloroethane	43 ± 2	7.3 ± 0.8
Alloxan + 1,1,2-trichloroethane	749 ± 383[b]	39.9 ± 20.8[b]
Control + corn oil	37 ± 1	5.9 ± 0.5
Alloxan + corn oil	56 ± 8	6.5 ± 1.1
Control + Trichloroethylene	51 ± 2	8.2 ± 0.9
Alloxan + Trichloroethylene	58 ± 7	14.8 ± 3.9
Control + 1,1,1-trichloroethane	42 ± 2	5.7 ± 0.5
Alloxan + 1,1,1-trichloroethane	65 ± 19	21.6 ± 13.1

[a] Rats were challenged ip with chloroform (0.5 ml/kg), 1,1,2-trichloroethane (0.05 ml/kg), trichloroethylene (1.0 ml/kg), or 1,1,1-trichloroethane (1.0 ml/kg) 3 days after single iv doses of alloxan (60 mg/kg). The animals were sacrificed 24 h later. Values expressed as mean ± SE ($n = 7$).

[b] Values significantly different ($P < 0.05$) from respective control group challenged with corn oil, chloroform, or 1,1,2-trichloroethane.

TABLE III. ALLOXAN PRETREATMENT ON THE LIVER INJURY INDUCED BY GALACTOSAMINE BERYLLIUM OR ANIT IN MALE RATS[a]

Challenge (response measured)	Treatment groups			
	Control + saline	Alloxan + saline	Control + challenge	Alloxan + challenge
Galactosamine (SGPT)	41 ± 2 (6)[b]	92 ± 18[c] (6)	232 ± 130 (6)	801 ± 264[d] (6)
Beryllium (ICDH)	9.7 ± 0.3 (3)	22.6 ± 5.8[c] (3)	21.5 ± 4.5[c] (8)	21.6 ± 5.1[c] (8)
ANIT (bilirubin)	0.1 ± 0.0 (6)	0.2 ± 0.1 (6)	1.7 ± 0.3[c] (6)	1.4 ± 0.3[c] (6)

[a] Rats were given galactosamine (200 mg/kg, iv), beryllium (0.25 mg/kg, iv) or ANIT (300 mg/kg, po), 3 days after single dose of alloxan (60 mg/kg, iv). The animals were sacrificed 24 hr later. SGPT activity is expressed in units/ml, ICDH in units/ml/min, and plasma bilirubin in mg/100 ml, mean ± SE.

[b] Numbers in parentheses are number of rats.

[c] Values significantly different ($P < 0.05$) from Control + saline.

[d] Values significantly different ($P < 0.05$) from Control + challenge.

alloxan-pretreated rats. The mean SGPT activity for alloxan-diabetic rats treated with insulin was only about one-sixth of that observed in alloxan-diabetic rats not treated with insulin.

In a similar experiment, treatment of alloxan-diabetic rats with a higher dosage of insulin 20 units/kg/day resulted in virtually a complete protection against the chloroform-induced elevation in SGPT activity (mean, 70 units/ml, $n = 2$, range 64–75). However, due to the high mortality produced by insulin alone under these conditions, further studies with insulin were curtailed.

The effects of alloxan on the liver injury produced by single intraperitoneal doses of trichloroethylene or 1,1,1-trichloroethane are given in Table I. Alloxan pretreatment had no effect on the SGPT activity of rats challenged with either of these halogenated hydrocarbons. Alloxan did produce apparent increases in the hepatic triglyceride concentrations of the challenged animals; however, due to the large variations encountered, these differences were not statistically significant.

Additional experiments were conducted to determine the effects of alloxan-induced diabetes on hepatotoxic agents with effects differing from those of the preceding haloalkane compounds. The challenging dose of each agent was selected at a level where some moderate degree of liver injury was produced in control (nonalloxan-pretreated) animals.

TABLE II. EFFECT OF INSULIN PRETREATMENT ON CHLOROFORM-INDUCED HEPATOTOXICITY IN ALLOXAN-DIABETIC RATS[a]

Treatment	Plasma glucose concn (mg/100 ml)		SGPT (units/ml)
	24 hr after Alloxan	At sacrifice	
Alloxan + chloroform	327 ±65	1040 ±135	5560 ±982
Alloxan + Insulin + chloroform	452 ±59	426 ±32[b]	943 ±625[b]

[a] Alloxan (60 mg/kg, iv) was given 3 days before chloroform (0.5 ml/kg, ip). Daily doses of protamine zinc insulin (10 units/kg/day, sc) began 24 hr after alloxan and continued for 3 days. The rats sacrificed 24 hr after the administration of chloroform. Values expressed as mean ± SE ($n = 5$).

[b] Values significantly different ($P < 0.05$) from group not given insulin.

The first of these agents tested was galactosamine, and SGPT activity was employed to assess liver damage. The elevations of SGPT activity in control animals challenged with galactosamine, was enhanced over threefold when this same challenging dose was given to alloxan-diabetic rats (Table III). Alloxan pretreatment alone caused only a slight increase in the serum enzyme.

Studies were also performed to determine

if alloxan pretreatment could potentiate the liver injury induced by beryllium when plasma ICDH activity was employed to assess liver damage. The ICDH activities following challenge with Be were similar in control and alloxan-pretreated rats (Table III). Alloxan alone was able to elevate ICDH activity to values comparable to that obtained when control or alloxan-diabetic rats were given Be.

In a last set of experiments we examined the effects of alloxan pretreatment on the hyperbilirubinemic response to ANIT (Table III). Alloxan, in the absence of ANIT, produced no effects on plasma bilirubin concentrations. The hyperbilirubinemia induced by ANIT in alloxan-diabetic rats was not significantly different from that observed when ANIT was administered to control rats.

Discussion. The present investigation has shown that alloxan pretreatment can potentiate the hepatotoxic response to several compounds and is thus not limited to the enhancement of CCl_4-induced liver injury previously reported (1). Our studies indicate that alloxan diabetes enhanced the hepatic injury induced by chloroform or 1,1,2-trichloroethane, which already possess fairly high hepatotoxic potentials. However, alloxan did not convert the mildly hepatotoxic agents, trichloroethylene or 1,1,1-trichloroethane, into potent hepatotoxic substances.

Traiger and Plaa (13) reported similar findings after examining the effects of isopropranol pretreatment on the potentiation of liver damage produced in mice by this same series of halogenated hydrocarbons. Plaa *et al.* (14) found that isopropanol pretreatment enhanced only the necrogenic responses of these compounds which act predominantly on the centrilobular cells of the liver; the necrogenic actions of Be and galactosamine were not increased by isopropanol pretreatment. With alloxan pretreatment the necrogenic response of rat livers to galactosamine, an agent which produces focal as well as periportal damage (4), was also enhanced. Therefore, the actions of alloxan which lead to the enhancement of chemically induced liver damage appear to be less specific than that of isopropanol.

Recently, Ilett *et al.* (15) reported that the liver injury induced by chloroform in mice may be mediated by the biotransformation of this compound to an active hepatotoxic intermediate. Pretreatment of laboratory animals with phenobarbital, an inducer of hepatic microsomal drug-metabolizing enzymes, is also known to potentiate the liver damage induced by each of the halogenated hydrocarbons studied in the present investigation (15–17).

Alloxan pretreatment enhances the activity of hepatic aniline hydroxylase in male rats (18, 19). However, in general, this diabetogenic agent reduces the activity of a number of hepatic microsomal drug-metabolizing pathways in male rats (18–21). Thus while metabolic activation of chloroform or 1,1,2-trichloroethane may perhaps play a role in their hepatotoxicity, it would seem questionable that this could serve to explain the potentiation of the liver injury produced by these halogenated hydrocarbons in alloxan-pretreated rats. At present, the mechanisms by which alloxan enhances the hepatotoxicity of chemicals are unknown.

The partial protection afforded by insulin against the liver injury induced by chloroform in alloxan-diabetic rats suggests that the potentiation observed with alloxan is dependent upon the diabetic state per se. The results of the present investigation, as well as those reported previously (1), indicate that experimentally induced diabetes can markedly alter the hepatotoxic responses to chemicals in rats.

Summary. Alloxan diabetes enhances the hepatotoxic response of male rats to chloroform and 1,1,2-trichloroethane, but not to trichloroethylene nor 1,1,1-trichloroethane. Insulin treatment partially protects the animals against the alloxan-induced enhancement of chloroform hepatotoxicity. Alloxan diabetes also enhances the hepatotoxic response to galactosamine but not to beryllium nor alpha-naphthylisothiocyanate.

The authors with to express their appreciation for the excellent technical assistance rendered by Mlles. Thérèse Vaillancourt, Suzanne Leroux, and Monique Morisset.

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Received February 3, 1975. P.S.E.B.M., 1975, Vol. 149.