Studies on the Protective Effect of Diphenylhydantoin Against Alloxan Diabetes in Mice[1] (36963)

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An earlier communication from these laboratories described the protective effect of diphenylhydantoin (DPH) against the diabetogenic action of alloxan in mice (1). The intraperitoneal injection of from 10 to 45 mg/kg of DPH, one hour prior to the intravenous injection of alloxan, prevents pancreatic *beta* cell necrosis and the development of chronic hyperglycemia.

Although the mechanism through which alloxan produces its pancreotoxic effect remains obscure, certain structural requirements have been recognized for this action. One of these requirements is the presence of an unsubstituted ureido group on the alloxan molecule. Alkyl substitutions on one or both of the nitrogens of alloxan results in decreased pancreotoxic activity (2). The DPH molecule also possesses an unsubstituted ureido moiety and this drug is known to influence pancreatic function (3–5). This structural similarity between alloxan and DPH has led us to hypothesize that DPH-induced protection against alloxan may be a reflection of a competition between the two compounds for the same binding site on the pancreatic *beta* cell (1).

An alternative explanation for the protective effect of DPH against alloxan is suggested by the results reported by Scheynius and Taljedal (6). These workers have shown that the intravenous injection of D-glucose prior to the administration of alloxan prevents the development of diabetes. Since DPH is known to produce hyperglycemia (1, 3–5) it is possible that the protective effect of DPH is mediated by this action.

The objectives of the experiments reported in this communication were to determine if the protective effect of DPH could be correlated to the hyperglycemic action of the drug, and the effect of alkyl substitution in the ureido group of DPH on the ability of the drug to prevent alloxan-induced diabetes.

Methods. Male albino mice (Laboratory Supply Co., Indianapolis) weighing 18 to 24 g were used in these experiments. The animals were housed in groups of ten with free access to food and water at all times.

Blood samples for glucose analysis were obtained by the orbital sinus puncture technique. Glucose analyses were performed by a glucose oxidase method (Beckman Glucose Analyzer). Since only small blood samples are required for this method (10 μl of serum) repeated samples were taken from the animals and each mouse served as its own control.

All drug solutions were prepared at concentrations which allowed for the administration of volume doses of 10 ml/kg. Alloxan monohydrate was dissolved in distilled water no more than 10 min prior to injection. Diphenylhydantoin sodium and D-glucose were dissolved in distilled water and 3-methyl diphenylhydantoin (3-M-DPH) was suspended in 10% Tween 80.

The intraperitoneal dosage levels of DPH and 3-M-DPH used in these experiments were selected on the basis of the anticonvulsant potency and time of peak activity in the supramaximal electroshock test (7). The anticonvulsant ED_{50} value of DPH was found to be 5.5 mg/kg with peak activity at one hour. Dosage levels tested for antagonism of alloxan diabetogenesis were 10 and 45 mg/kg administered one hour prior to alloxan. The anticonvulsant ED_{50} value of 3-M-DPH was found to be 22.5 mg/kg with peak activity

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TABLE I. Effects of Glucose and Diphenylhydantoin on the Development of Alloxan-Induced Diabetes in Mice.[a]

Treatment regimen	Mean blood glucose levels (mg/100 ml ± S.E.M.)			No. diabetic[d]
	Pretreatment[b]	Pre-alloxan[c]	72 hr post alloxan	No. tested at 72 hr
Saline + Saline	171 ± 7	194 ± 15	154 ± 5	0/5
Saline + Alloxan	150 ± 18	163 + 14	711 ± 24[e]	5/5
DPH (45 mg/kg) + Alloxan	161 ± 15	298 ± 27[e]	106 ± 6[f]	0/5
DPH (10 mg/kg) + Alloxan	160 ± 5	201 ± 14[e]	152 ± 17	0/5
Glucose (1.0 g/kg) + Alloxan	169 ± 8	508 ± 29[e]	169 ± 21	0/5
Glucose (0.2 g/kg) + Alloxan	149 ± 16	234 ± 9[e]	339 ± 20[e]	4/5

[a] Glucose (1.0 or 0.2 g/kg) was administered intravenously 5.0 min prior to the intravenous injection of 75 mg/kg alloxan. DPH (45 or 10 mg/kg) was administered intraperitoneally 60 minutes prior to the intravenous injection of 75 mg/kg of alloxan.

[b] Blood samples taken immediately prior to administration of saline, glucose or DPH.

[c] Blood samples taken 1.0 min prior to administration of alloxan or saline.

[d] Animals considered to be diabetic if blood glucose exceeded 300 mg/100 ml.

[e] Significantly greater than pretreatment level ($p < 0.05$).

[f] Significantly less than pretreatment level ($p < 0.05$).

at 4 hr. The dosage level tested for antagonism of alloxan was 45 mg/kg administered either four or one hour prior to alloxan. The intravenous dosage level of alloxan was 75 mg/kg.

Differences in blood glucose levels between treatment groups were evaluated using the Student *t* test.

Results. The comparative protective effects of DPH and D-glucose against alloxan diabetogenesis are shown in Table I. In this experiment blood glucose levels were determined immediately prior to the administration of either saline, D-glucose or DPH. The second sample was taken one minute prior to the intravenous injection of alloxan (or saline in the case of controls). The final blood sample was taken 72 hr after the injection of alloxan.

It can be seen that the administration of alloxan alone, as expected produced a marked hyperglycemia at the 72-hr interval. The mean blood glucose level of this group was 711 mg/100 ml with a range of 642 to 787 mg/100 ml. Mice which had been pretreated with either the 45 or 10 mg/kg dose of DPH did not exhibit hyperglycemia at the 72-hr interval. In fact, animals which had received the higher dose of DPH had significantly lower blood glucose levels than they had before treatment or than the saline–saline controls. This phenomenon has been reported before (1) and there is no ready explanation for its occurrence.

Pretreatment of mice with 1.0 g/kg of D-glucose also afforded complete protection against the development of alloxan-induced diabetes. The mean blood glucose level of this group at the 72-hr interval was identical to the pretreatment level. Pretreatment of mice with the 0.2 g/kg dose of D-glucose afforded partial protection against alloxan-induced diabetes. The mean blood glucose level of this group 72 hr after alloxan was not as great as that observed iin animals that had received alloxan alone but it was significantly greater than that of the saline–saline controls or of the values observed in the same animals before treatment.

Immediately prior to the administration of alloxan all pretreated mice had significantly elevated blood glucose levels. However, the magnitude of the hyperglycemia noted after the administration of 0.2 g/kg of glucose, a treatment which protected only 1 out of 5 mice from alloxan, was slightly greater than that produced by the 10 mg/kg dose of DPH which afforded complete protection. Also, when pre-alloxan glucose levels are compared to pretreatment levels, the administration of the 0.2 g/kg dose of glucose produced a significantly greater elevation than did the 10 mg/kg dose of DPH (mean increase of 40 ± 10 mg/100 ml for DPH *vs* 84 ± 13 mg/100 ml for glucose, $p < 0.05$).

TABLE II. Effect of Diphenylhydantoin and 3-Methyl Diphenylhydantoin on the Development of Alloxan-Induced Diabetes in Mice.[a]

Treatment	Pretreatment interval (hr)	Mean blood glucose (mg/100 ml) Hours after Alloxan 0	0.75	24
Alloxan alone	—	220 ± 108	580 ± 77	579 ± 87
DPH + Alloxan	1.0	217 ± 7	560 ± 33	198 ± 29[b]
Alloxan alone	—	166 ± 12	575 ± 26	633 ± 36
3-M-DPH + Alloxan	1.0	174 ± 10	563 ± 39	573 ± 19
Alloxan alone	—	134 ± 9	516 ± 47	590 ± 48
3-M-DPH + Alloxan	4.0	155 ± 10	540 ± 26	567 ± 58

[a] DPH or 3-M-DPH was administered intraperitoneally (45 mg/kg) prior to the intravenous injection of 75 mg/kg of alloxan.

[b] Significantly less than alloxan alone ($p < 0.001$).

The comparative effects of DPH and 3-M-DPH pretreatment on alloxan diabetogenesis are shown in Table II. In these experiments blood samples were obtained immediately prior to the administration of DPH or 3-M-DPH, then 45 min and 24 hr after the injection of alloxan. Both anticonvulsants were administered at dosage levels of 45 mg/kg, ip.

It can be seen from these data that again, DPH, used in this experiment as a positive control, afforded complete protection against alloxan. The 3-methyl analog of DPH on the other hand, was without effect on the development of chronic hyperglycemia regardless of the pretreatment interval employed.

Discussion. Increased levels of blood glucose are known to protect mice against alloxan-induced diabetes (6). In spite of the fact that DPH produces hyperglycemia in mice it is unlikely that this effect accounts for the protective action of the anticonvulsant against alloxan diabetogenesis. An intravenous dose of 0.2 g/kg of glucose afforded only partial protection against alloxan but caused a greater elevation in blood glucose levels than did the 10 mg/kg dose of DPH which afforded complete protection.

The comparative effects of DPH and 3-M-DPH on alloxan diabetogenesis lends preliminary support to the possibility that the ureido portion of the DPH molecule is involved in the pancreatic action. At dosage levels which are pharmacologically equal ($2 \times$ anticonvulsant ED_{50}) DPH but not 3-M-DPH prevents the development of alloxan diabetes. Since the ureido portion of the alloxan molecule appears to be important for its effect on the pancreas (2) it is possible that DPH and alloxan compete for the same binding site on the *beta* cell membrane.

Diphenylhydantoin is known to influence pancreatic function. Levin *et al.* (5) have shown that DPH inhibits glucose-stimulated insulin secretion from the isolated perfused rat pancreas. Similarly, Kizer *et al.* (8) have shown that DPH blocks glucose, tolbutamide and methacholine-induced insulin secretion from isolated pancreatic pieces. In our own laboratories we have demonstrated that pharmacological doses of DPH antagonize the hypoglycemic action of tolbutamide (9). Interestingly, tolbutamide also possesses a ureido moiety in its molecule.

It is recognized that the present studies on DPH and its 3-methyl analog represent only indirect evidence to support our hypothesis. Several factors, such as absorption, metabolism and distribution of 3-M-DPH may account for the inability of this agent to prevent alloxan-induced diabetes. Indeed, Kizer *et al.* (8) have reported that mephenytoin, and anticonvulsant hydantoin which has a methyl group in the number 3 position of the hydantoin ring, inhibits glucose stimulated insulin secretion from isolated pancreatic pieces. We are currently conducting *in vitro* studies to determine the interactions between alloxan, DPH and 3-M-DPH on insulin secretion by isolated pancreatic islets.

Summary. The protective effects of D-glucose and diphenylhydantoin (DPH) against alloxan-induced diabetes have been compared.

Diphenylhydantoin, at intraperitoneal doses of either 10 or 45 mg/kg and D-glucose at an intravenous dose of 1.0 g/kg were found to afford complete protection against alloxan. An intravenous dose of 0.2 g/kg of D-glucose afforded only partial protection. Since the lower dose of D-glucose produced a greater increase in blood glucose than did the lower dose of DPH, it was concluded that the protective effect of DPH against alloxan can not be explained solely on the basis of the DPH-induced hyperglycemia. The 3-methyl analog of DPH was found to be ineffective in blocking the diabetogenic effect of alloxan. This finding suggests that the interaction between DPH and alloxan may be a reflection of a competition between the two agents for a common binding site on the pancreatic *beta* cell.

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