Involvement of Blood Glucose in the Dimethylthiourea-Induced Protection against Alloxan-Induced Diabetes

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ABSTRACT—Dimethylthiourea (DMTU, 4.0 mmol/kg) injected into mice 30 min prior to alloxan injection markedly protected mice against the diabetogenic actions of 75 mg/kg alloxan. At 30 min after the above dose of DMTU alone (no alloxan), there was a marked rise in blood glucose. Mannoheptulose, an antagonist of glucose action at pancreatic β-cells, when given 24 min after DMTU and 6 min before alloxan, eliminated the DMTU-induced protection. The protection was also removed in the fasted mice in which DMTU did not cause hyperglycemia. These results indicate that DMTU protected mice from alloxan-induced diabetes by the indirect mechanism of producing hyperglycemia at the time of alloxan injection.

Keywords: Dimethylthiourea, Hyperglycemia, Diabetes (alloxan-induced)

Of commonly used radical scavengers, dimethylthiourea (DMTU) was the most potent scavenger of hydroxyl radicals in vitro and was suggested to be useful for investigating the role of hydroxyl radicals in vivo (1, 2). The cytotoxicity of alloxan is suggested to be mediated through oxygen-containing radicals, particularly hydroxyl radicals (3-5), and a number of compounds bearing a high reactivity towards hydroxyl radicals, given to mice before alloxan, have been reported to prevent the diabetogenic actions of alloxan (6-10). However, it has been shown that interpretation of the results of in vivo experiments is complicated by the possibility that the scavengers may protect by indirect or unknown mechanisms and not by their ability to react with hydroxyl radicals. According to Schauberger et al. (11), while *n*-butanol, the most effective hydroxyl radical scavenger in vitro among various aliphatic short chain alcohols (6), had the highest potency in producing protection from alloxan diabetes, its protection was based on the indirect mechanism of producing hyperglycemia at the time of alloxan injection.

The present study was carried out to examine the effect of DMTU on the alloxan-induced diabetes and the possibility that it acts via an indirect mechanism.

The animals used were male 6-week-old ddY mice weighing about 30 g, which were obtained from Japan SLC, Inc., Hamamatsu. DMTU and alloxan monohydrate were obtained from Wako Pure Chemical Industries, Osaka. Mannoheptulose was from Sigma Chemical Company, St. Louis, MO, USA. Other reagents used were of analytical grade.

Food and water were withheld from the mice 3-4 hr prior to alloxan injection. Alloxan at 75 mg/kg was injected intravenously (tail vein) into the mouse; the alloxan was prepared in cold isotonic saline and kept on ice prior to injection. One hour after alloxan, the mice were given free access to food and water. The extent of hyperglycemia at 72 hr after alloxan injection was used as an index of alloxan-induced damage. Pretreatment with DMTU (dissolved in saline) was given intraperitoneally 30 min before alloxan. Blood was collected by decapitation between 14:00-15:00, and blood glucose was measured by a glucose oxidase method (Blood Sugar-GOD-Perid-test, Boehringer Mannheim Yamanouchi, Tokyo). Data are shown as the mean±S.E.M. Comparisons of the mean values were made by analysis of variance followed by Duncan's multiple range test.

Alloxan injection to mice at 75 mg/kg caused a significant rise in blood glucose at 72 hr. Mice pretreated with DMTU at the dose of 4 mmol/kg 30 min prior to alloxan were largely protected against the diabetogenic actions of alloxan (Table 1). As to the incidence rate of diabetes, all of mice in the alloxan control group had blood glucose values greater than 200 mg/100 ml, while only one of 10 mice in the DMTU plus alloxan group had values over 200 mg/100 ml.

In separate experiments, when blood glucose was measured 30 min after DMTU (4 mmol/kg) alone (no alloxan), there was a marked rise in blood glucose as compared

Table 1. Protection by DMTU against the diabetogenic action of alloxan in mice

Treatment			Blood glucose (mg/100 ml)	Incidence[#]
Saline		Saline	134.8± 4.1**	0/10
Saline		Alloxan	377.8±20.6	10/10
DMTU	1.0 mmol/kg	Alloxan	328.0±50.3	6/10
	2.0	Alloxan	342.2±37.7	8/10
	4.0	Alloxan	167.7±23.0**	1/10

DMTU was injected intraperitoneally 30 min before alloxan (75 mg/kg, i.v.). Blood glucose was measured at 72 hr after alloxan. [#]: Positive for over 200 mg/100 ml of blood glucose. Significantly different from the alloxan control (**$P<0.01$). Each value represents the mean±S.E.M. of the results of 10 mice.

Table 2. Effect of mannoheptulose (MH) on the protection by DMTU against alloxan-induced diabetes

Treatment			Blood glucose (mg/100 ml)	Incidence[#]
Saline	Saline	Saline	151.9± 3.7**	0/8
Saline	Saline	Alloxan	375.8±40.2	7/8
Saline	MH	Alloxan	442.8±32.9	8/8
DMTU	Saline	Alloxan	154.6±15.6**	1/8
DMTU	MH	Alloxan	419.3±13.5	8/8
DMTU	MH	Saline	135.1± 5.2**	0/8

MH (4.0 g/kg) was injected intravenously 24 min after DMTU (4.0 mmol/kg, i.p.) and 6 min before alloxan. Blood glucose was measured at 72 hr after alloxan. [#]: Positive for over 200 mg/100 ml of blood glucose. Significantly different from the alloxan control (**$P<0.01$). Each value represents the mean±S.E.M. of the results of 8 mice.

to the control group (control=150.0±3.7, N=6 vs. DMTU=231.8±12.9, N=6, $P<0.01$). However, the lower doses of DMTU that could not protect mice from alloxan-induced diabetes did not elevate blood glucose as well (control=150.7±3.7, N=6; DMTU, 1.0 mmol/kg=150.3±3.7, N=6; 2.0 mmol/kg=183.1±20.7, N=6). These results showed that mice showing the anti-diabetogenic effect by DMTU exhibited hyperglycemia at the time of the alloxan injection. The administration of glucose prior to alloxan was reported to prevent mice from alloxan-induced diabetes (12, 13). The protection against alloxan toxicity may result from a conformational change in the pancreatic β-cell membrane by glucose, glucose transport or glucose metabolism (12). Therefore, the hyperglycemia caused by DMTU is expected to protect mice from alloxan toxicity.

Mannoheptulose is known to eliminate the protection from alloxan-induced diabetes afforded by glucose (12, 13). According to Rossini et al. (14), when animals were injected simultaneously with alloxan and mannoheptulose, the amount of alloxan required to provoke a given degree of hyperglycemia was decreased in a dose-related manner as the amount of mannoheptulose injected was increased. So, mannoheptulose is likely to sensitize the β-cells to alloxan, presumably by removing the protection of endogenous circulating glucose. If the hyperglycemia resulting from DMTU injection protects animals from alloxan, injection of mannoheptulose is assumed to eliminate this protective effect. The possibility was tested by the intravenous injection of mannoheptulose 24 min after DMTU and 6 min before alloxan. Mannoheptulose intensified the diabetogenic action of alloxan in the control group, and it completely eliminated the protection against alloxan-induced diabetes in the DMTU-pretreated mice (Table 2). The coadministration of DMTU with mannoheptulose without alloxan did not alter blood glucose at 72 hr.

Furthermore, DMTU-induced increase in blood glucose was largely suppressed in the mice fasting for 24 hr prior to DMTU injection, although there was a little difference between the control and DMTU group (control=84.0±9.3, N=6 vs. DMTU=96.3±4.7 mg/100 ml, N=6, $P<0.05$). When DMTU was injected to fasted mice 30 min prior to alloxan, the DMTU-induced protection against alloxan-induced diabetes produced in the fed mice was completely abolished (control=442.6±9.3, N=8 vs. DMTU=443.6±9.7 mg/100 ml, N=8). These results indicate that the DMTU-induced increase in blood glucose at the time of alloxan injection involves the anti-diabetogenic action of DMTU.

According to Tibaldi et al. (10), since protection of mice from alloxan provided by various urea derivatives (monomethylurea, monoethylurea and diethylurea) did not correlate with the degree of the increased blood glucose produced by the injection of the derivatives, but correlated instead with that of their in vitro scavenging capacity of hydroxyl radicals, their anti-diabetogenic effects may be due to their scavenging capacity of hydroxyl radicals. However, the possible involvement of the increased blood glucose could not necessarily be ruled out, because they did not evaluate the presence of antagonism by mannoheptulose against the urea derivative-induced protection. Furthermore, there is no direct evidence that alloxan is involved in the production of hydroxyl radicals in vivo and that the known hydroxyl radical scavengers protect pancreatic β-cells by scavenging the radicals derived from alloxan. In the present study, the development of the anti-diabetogenic effect with DMTU was found to parallel the difference in the variation of blood glucose at the time of alloxan injection. So, to evaluate whether DMTU protects mice from alloxan by an indirect mechanism, evidence was obtained using the following two experimental

approaches: First, mannoheptulose, an agent that antagonizes the action of glucose at pancreatic β-cells, completely eliminated the protection afforded by DMTU. Second, DMTU-induced protection was also removed in the fasted mice in which the DMTU-induced increase of blood glucose was markedly suppressed. Under the above two experimental conditions, DMTU did not exhibit any anti-diabetogenic activity. Therefore, the present results are reasonably explained by the hyperglycemia produced by DMTU, although the possibility could not be excluded that its scavenging of hydroxyl radicals may be partially involved in the DMTU-induced protection against alloxan diabetes. In conclusion, the present evidence indicate that DMTU-induced hyperglycemia is responsible for the protection from alloxan diabetes in mice.

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