Protection by dimethyl urea against hyperglycaemia, but not insulitis, in low-dose streptozotocin-induced diabetes in the mouse

S. Sandler

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

Summary. The protective effect of dimethyl urea, a hydroxyl radical scavenger, against low-dose streptozotocin-induced diabetes has been evaluated. Dimethyl urea was given to C57BL/KsJ mice before five daily injections of streptozotocin. The saline pre-treated control animals became gradually hyperglycaemic, whereas the dimethyl urea treated group remained normoglycaemic during the 11 week follow-up period. Two weeks after the first streptozotocin injection, six out of ten dimethyl urea-treated and 12 out of 15 saline-treated mice had insulitis. Four or 11 weeks after the streptozotocin treatment, insulitis was rare in both groups. Multiple injections of dimethyl urea only did not affect the serum glucose concentrations or the islet morphology. It is suggested that dimethyl urea protected against hyperglycaemia by reducing the β-cell cytotoxic effects of the low doses of streptozotocin. An increased number of cells would thus be preserved and the animals less prone to develop diabetes, despite the presence of an inflammatory process in the pancreatic gland.

Key words: Dimethyl urea, C57BL/KsJ mice, insulitis, pancreatic islets, streptozotocin.

One animal model for human insulin-dependent diabetes that has attracted a great deal of interest during recent years is the multiple streptozotocin (STZ)-induced diabetes in the mouse originally described by Like and Rossini [1]. This model is characterized by both a gradual development of hyperglycaemia and a round cell infiltration of the pancreatic islets leading to marked insulitis.

Although an autoimmune component seems to be involved in the aetiology of multi-STZ diabetes, the direct cytotoxic effects of STZ must be kept in mind. In line with this, compounds known to reduce STZ β cell-cytotoxicity would be worthwhile testing. Indeed, it has been found that 3-0-methyl-glucose and nicotinamide attenuate the diabetic syndrome induced by multiple STZ injections [2]. Recent investigations have suggested that free oxygen radicals may participate in STZ β cell injury [3–8]. However, Gold et al. did not observe any influence on the diabetic state induced by multiple STZ doses by pre-treatment with the superoxide anion scavenging enzyme superoxide dismutase [9].

In the present study the effect of pre-treatment with the hydroxyl radical scavenger dimethyl urea (DMU) before multiple low doses of STZ on the development of hyperglycaemia and pancreatic insulitis have been examined. DMU was recently found partly to protect against diabetes induced by a single diabetogenic dose of STZ [5].

Materials and methods

Animals

Inbred male C57BL/KsJ mice, originally obtained from the Jackson Laboratory, Bar Harbor, Maine, USA, aged 12–16 weeks, were used. The animals were allowed free access to tap water and laboratory chow (Ewos, Anticimex, Type R 3, Södertälje, Sweden) throughout the experiments.

Treatment

The mice were pretreated 'intraperitoneally with either DMU-solution (20 °C; 4 g/kg body weight; Sigma Chemicals, St. Louis, Missouri, USA) or an equal volume of saline (9 g/l, 500 µl) 30 min before the daily injections of STZ. STZ (kindly provided by Dr. W.E. Dulin; lot 60, 273-5 U-9889, Upjohn, Kalamazoo, Michigan, USA) was dissolved immediately before use in cold citrate buffer (10 mmol/l, pH 4.5) and given intraperitoneally (40 mg/kg body weight, 200 µl) for 5 consecutive days. Blood samples from the non-fasted animals were collected once or every second week without anaesthesia by retroorbital sinus puncture. Serum glucose was assayed using an automated analyser (Glucose Analyzer 2, Beckman Instruments, Fullerton, California, USA). In a series of control experiments, the animals were given multiple DMU or saline injections only, 30 min before intraperitoneal injections of citrate buffer.
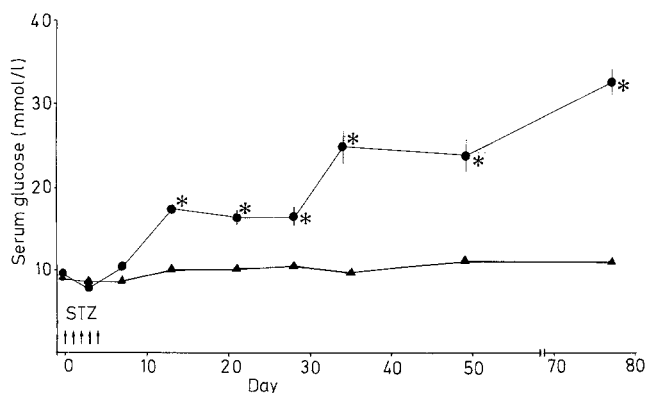
Fig. 1. Serum glucose values (mean ± SEM when exceeding 10% of the mean) for C57BL/KsJ mice given five daily injections of streptozotocin (40 mg/kg body weight, intraperitoneally) pre-treated with either dimethyl urea (▲——▲ : 4 g/kg body weight, intraperitoneally) or saline (●——● : 9 g/l; 500 μl, intraperitoneally). * $p < 0.001$ when using unpaired t-test

Table 1. Pancreatic islet morphology at different times after the first of five multiple sub-diabetogenic doses of streptozotocin in C57BL/KsJ mice pre-treated with either dimethyl urea or saline

	Islet morphology rank			
	A	B	C	D
Dimethyl urea + streptozotocin				
14 days ($n = 10$)	2	2	6	0
21 days ($n = 4$)	0	2	2	0
28 days ($n = 22$)	19	3	0	0
77 days ($n = 7$)	6	0	1	0
Saline + streptozotocin				
14 days ($n = 15$)	2	1	12	0
21 days ($n = 5$)	1	0	3	1
28 days ($n = 17$)	4	2	0	11
77 days ($n = 9$)	1	0	0	8

A: Essentially normal islet morphology; B: slight mononuclear cell infiltration in the peri-insular tissue; C: cell infiltration into a majority of the islets (insulitis); D: diabetic appearance with only a few remaining small and partially disintigrated islets with pycnotic nuclei

Morphology

The animals were killed by cervical distension after 14, 21, 28 and 77 days and the pancreases were removed quickly, fixed in Bouin's solution and embedded in paraffin. Sections (7 μm thickness) were cut and stained with hematoxylin eosin. The islet morphology was examined by a light microscope (Leitz, Wetzlar, FRG), with the examiner unaware of the origin of the pancreatic sections. The islet morphology was ranked according to four arbitrary classes by light microscopy [5]. (A) essentially normal islet morphology; (B) slight mononuclear cell infiltration in the peri-insular tissue; (C) cell infiltration into a majority of the islets, i.e. insulitis; (D) diabetic appearance with only a few remaining small and partially disintigrated islets with pycnotic nuclei.

Statistical analysis

Groups of data were compared using Student's t-test.

Results

There was a gradual increase in the serum glucose concentrations of the multi-STZ-treated mice pretreated with saline during the 11-week follow-up period (Fig. 1). In contrast, the animals given DMU and STZ treatment remained normoglycaemic throughout the whole observation period. Since the serum glucose concentrations of the DMU-treated animals did not increase, some of these mice were sacrificed after 4 instead of 2 weeks to ascertain a possible delay in the development of hyperglycaemia.

In control experiments, animals given five multiple doses of DMU or saline before citrate injections remained normoglycaemic. The serum glucose concentrations of the DMU-treated animals were 6.9 ± 0.2 ($n = 13$), 7.8 ± 0.3 ($n = 13$) and 8.0 ± 0.3 ($n = 7$) mmol/l and the saline-treated animals 8.3 ± 0.2 ($n = 12$), 8.0 ± 0.2 ($n = 12$) and 8.0 ± 0.3 ($n = 6$) on days 7, 14 and 21, respectively. No inflammatory reactions were observed in the pancreatic glands.

The results of the morphological examinations of the STZ-treated animals are summarized in Table 1. The highest frequency of insulitis in both groups was observed 14 days after starting the STZ regimen. Six out of ten DMU-treated and 12 out of 15 saline-treated animals were classified as having insulitis. A similar incidence of insulitis was found in the pancreatic glands after 3 weeks. Animals sacrificed after 4 and 11 weeks showed virtually no signs of insulitis. A majority of the saline pre-treated mice at that time displayed diabetic-like pancreatic morphology with only a few non-infiltrated islets remaining in the glands (Class D). Often pancreatic sections from both categories of animals obtained during weeks 2 and 3 after STZ injections exhibited lymph nodes within the pancreatic parenchyma and in the periglandular fat tissue.

Discussion

The present finding that pre-treatment with a drug that reduced the β-cell toxic action of STZ [5, 8], protected against multi-STZ induced hyperglycaemia, suggests that direct STZ cytotoxicity is of importance for the development of diabetes in the multi-STZ model. This is in agreement with the findings of Bonnevie-Nielsen et al. that a major loss of β cells and a concomitant decrease of insulin release capacity precede hyperglycaemia, as well as the peak incidence of insulitis [10].

The fact that a large proportion of the DMU-treated animals sacrificed on days 14 and 21 exhibited insulitis, but remained normoglycaemic, may suggest that the inflammatory reaction is not directed against the β cells. Thus, the role of insulitis in the development of hyperglycaemia in this animal model may be insignificant as suggested previously [11, 12]. It could be also that a direct cytotoxic action of STZ is a prerequisite for induction of insulitis [2]. Animals given a drug (DMU) that re-

duces repeated minor STZ cytotoxic effects, would be less susceptible to suffer a diabetogenic immune reaction.

In a previous investigation, we found that multi-STZ hyperglycaemic mice, which had been cured with syngeneic islets implanted intrasplenically, reverted to hyperglycaemia when given three small doses of STZ. Similarly, cured animals rendered diabetic by a single STZ injection remained normoglycaemic [13]. These results suggested that the multi-STZ treatment had induced immune reactions directed against the β cells when exposed to STZ. Thus, it may be that when these animals were re-exposed to a low STZ dose, an immune response was elicted of such magnitude that hyperglycaemia developed. It has been proposed that human diabetes may result from cumulative β-cell damage induced by sequential environmental insults by toxic chemicals and infectious virus [14]. A few reports have also presented suggestive data that chemical agents may possess a diabetogenic action in man [15, 16]. It remains to be shown that these noxious agents are of importance in human diabetes. If so, prophylactic treatment of individuals who might be expected to develop insulin-dependent diabetes mellitus using free radical scavengers could be considered.

References

1. Like AA, Rossini AA (1976) Streptozotocin-induced pancreatic insulitis: a new model of diabetes mellitus. Science 193: 415–417
2. Rossini AA, Like AA, Chick WL, Appel MC, Cahill Jr GF (1977) Studies on streptozotocin-induced insulitis and diabetes. Proc Natl Acad Sci USA 74: 2485–2489
3. Robbins MJ, Sharp RA, Slonim AE, Burr IM (1980) Protection against streptozotocin-induced diabetes by superoxide dismutase. Diabetologia 18: 55–58
4. Marklund S, Grankvist K (1980) Polyethyleneglycol-superoxide dismutase (PEG-SOD) protect against streptozotocin-induced diabetes in mice. Acta Endocrinol 98: 43 (Abstract)
5. Sandler S, Andersson A (1982) The partial protective effect of the hydroxyl radical scavenger dimethyl urea on streptozotocin-induced diabetes in the mouse in vivo and in vitro. Diabetologia 23: 374–378
6. Gandy SM, Buse MG, Crouch RK (1982) Protective role of superoxide dismutase against diabetogenic drugs. J Clin Invest 70: 650–658
7. Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM (1983) Modification of chemically induced diabetes in rats by vitamin E. Supplementation minimizes and depletion enhances development of diabetes. J Clin Invest 71: 1282–1288
8. Sandler S, Welsh M, Andersson A (1983) Streptozotocin-induced impairment of islet B-cell metabolism and its prevention by a hydroxyl radical scavenger and inhibitors of poly(ADP-ribose)synthetase. Acta Pharmacol Toxicol 53: 392–400
9. Gold G, Manning M, Heldt A, Nowlain R, Pettit JR, Grodsky GM (1981) Diabetes induced with multiple subdiabetogenic doses of streptozotocin. Lack of protection by exogenous superoxide dismutase. Diabetes 30: 634–638
10. Bonnevie-Nielsen V, Steffes MW, Lernmark Å (1981) A major loss in islet mass and B-cell function precedes hyperglycaemia in mice given multiple low doses of streptozotocin. Diabetes 30: 424–429
11. Leiter EH (1982) Multiple low-dose streptozotocin-induced hyperglycemia and insulitis in C57BL mice: influence of background, sex and thymus. Proc Natl Acad Sci USA 79: 630–634
12. Prowse SJ, Steele EJ, Lafferty KJ (1982) Islet allografting without immunosuppression: reversal of insulitis-associated diabetes and a case of spontaneous juvenile onset diabetes in mice. Aust J Exp Biol Med Sci 60: 619–627
13. Sandler S, Andersson A (1981) Islet implantation into mice with pancreatic insulitis. Acta Path Microbiol Scand, Sect A 89: 107–112
14. Toniolo A, Onodera T, Yoon J-W, Notkins AL (1980) Introduction of diabetes by cumulative environmental insults from viruses and chemicals. Nature 288: 383–385
15. Karam JH, Lewitt PA, Young CW, Nowlain RE, Frankel BJ, Fujiya H, Freedman RZ, Grodsky GM (1980) Insulinopenic diabetes after rodenticide (Vacor) ingestion. A unique model of acquired diabetes in man. Diabetes 29: 971–978
16. Helgason T, Jonasson MR (1981) Evidence for a food additive as cause of ketosis-prone diabetes. Lancet 2: 716–720

Dr. Stellan Sandler
Department of Medical Cell Biology
Biomedicum
P. O. Box 571
S-751 23 Uppsala
Sweden