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Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals

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Summary. A group of 36 diabetes-prone BB Wistar rats were given prophylactic insulin treatment with heat-treated bovine ultralente insulin (15 IU · kg^{-1} · day^{-1}) from 50 to 142 days of age. The incidence of Type 1 (insulin-dependent) diabetes at the end of the treatment period was compared to that of 36 control animals given insulin only from the first day of glycosuria. At withdrawal of the prophylactic insulin treatment, 6 of 36 treated animals were insulin-dependent, while 15 of 36 control animals had developed diabetes ($p < 0.02$). One control animal (day 153) and 2 insulin-treated animals (day 172 and 186) subsequently developed diabetes, yielding an overall diabetes incidence of 16 of 36 controls against 8 of 36 insulin treated ($p < 0.05$). The finding that prophylactic insulin treatment of diabetes-prone BB Wistar rats reduced the incidence of diabetes suggests a relationship of the immune destruction of B cells to the endogenous insulin production/secretion rate.

Key words: BB rat, insulin-dependent diabetes, insulin therapy, pathogenesis, prophylaxis, long-acting insulin.

The development of diabetes in colonies of the spontaneous diabetic BB Wistar rats is mainly determined by the genetic predisposition coupled to the $RT1^u$ haplotype and T-cell abnormality [1]. Whether an animal becomes diabetic or not may, however, be under the influence of environmental factors, nutrition and stress. For example, it has been shown that feeding prediabetic diabetes-prone BB Wistar rats defined diets reduced the incidence of diabetes [2, 3]. Moreover, variations in diet composition or food restriction have significantly reduced the need for insulin in overtly diabetic BB Wistar rats [4]. In streptozotocin-treated neonatal rats, the administration of exogenous insulin for 4 days reduced the B cell damage and improved the recovery of insulin stores [5]. The findings of this study suggest that administration of exogenous insulin to BB rats during the prediabetic period could render the B cells less vulnerable to immune aggression.

Materials and methods

Animals and rearing conditions

The ancestors of the BB Wistar rats used in the present study were obtained from Dr. Thibert, Animal Resources Division, Health Protection Branch, Health & Welfare Canada, Ottawa, Ontario, Canada. In three out of the four litters supplied, Type 1 (insulin-dependent) diabetes mellitus developed. Only animals from those three litters were used for breeding. The colony was maintained by random mating of diabetic males with females from high incidence litters (diabetic sibling, diabetic outcross, non-diabetic sibling or non-diabetic outcross). Animals were bred and kept under similar conditions in plastic cages with wood shavings. Brood Stock Feed for Rats and Mice (R3) and citrated tap water were available ad libitum throughout the experiment. The animal cages were kept in laminar flow units to reduce the risk of infection. The room was kept at 21 °C and 50% relative humidity with lights on from 6.00 to 18.00 h.

Standard diabetes care

From 40 days of age, animals were weighed 3 times a week and urine tested for glucose and ketones with Keto-Diabur-Teststrips (Boehringer-Mannheim, Mannheim, FRG) until the first day of a positive glucosuria test, then every working day subsequently. Diabetes was considered present from this day on if the animals subsequently showed a stable or increased insulin need. Diabetic animals were given subcutaneous injections in the neck region of a special heat-treated bovine ultralente insulin preparation which could control hyperglycaemia for nearly 24 h in rats [6, 7]. Before injection, the insulin suspension was diluted with the suspension medium to 10 IU/ml. The overall incidence of diabetes among animals surviving more than 180 days was 55%, with onset at 101 ± 24 days (SD, $n = 54$) for males and 101 ± 17 days (SD, $n = 48$) for females.

Experimental procedure

Two pilot studies were carried out to determine a safe dose of insulin. In the first groups of normal Wistar rats, 5 males and 5 females, 50 days of age, were given heat-treated bovine ultralente insulin 0, 8,

Table 1. Cause of deaths among control and prophylactically insulin-treated BB Wistar rats

	Sex	Died day[a]	Type 1 Diabetes duration days	History	
Control group	M	47	37		Killed for fusion of splenocytes
	M	59	35	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	M	60	13	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	F	60	13	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	M	61	11	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	F	65	32	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	F	65	41	Weight loss, hyperglycaemia & ketosis	Insufficient insulin therapy
	F	103	95	Noisy respiration	Pneumonia
	F	154	108	Weight loss; decreasing insulin, hypoglycaemia	Hyperinsulinization
	M	171	69	Blindness	No other abnormalties
	F	172	107	Blindness	No other abnormalties
Prophylactic insulin group	F	60	?	Weight loss, hyperglycaemia & ketosis	Diabetic, insufficient insulin
	F	61	?	Weight loss, hyperglycaemia & ketosis	Diabetic, insufficient insulin
	F	68	?	Weight loss, hyperglycaemia & ketosis	Diabetic, insufficient insulin
	M	71	-	Normoglycaemia, weight loss	Multiple small gastric ulcers
	F	86	-	-	Oophoritis
	M	163	41	Mated 1 day before death	No abnormalities
	F	165	-	Gradual weight gain, then weight loss	Massive abdominal lymphomas
	F	172	-	Noisy respiration	Pneumonia

[a] From day 1 of prophylactic insulin injection

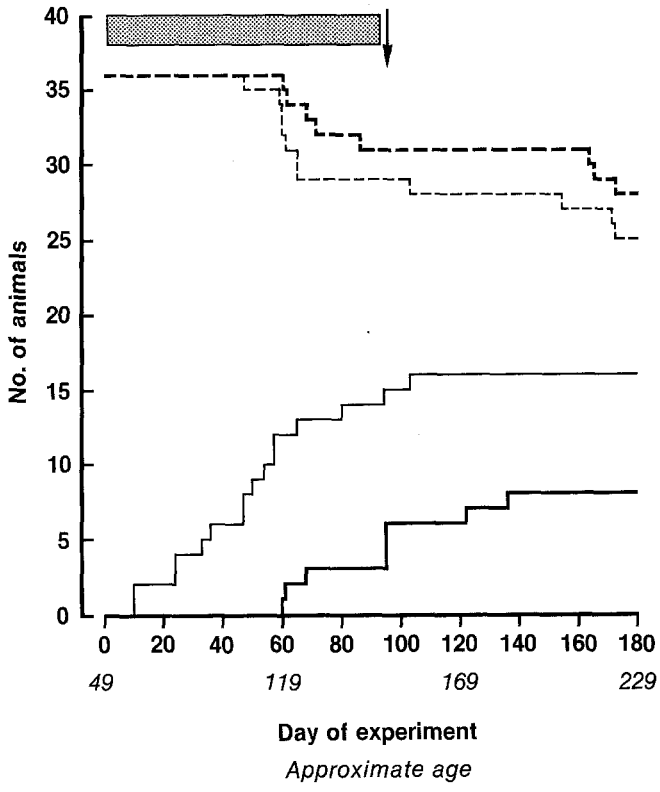

Fig. 1. Accumulated diabetes incidence in prophylactically insulin-treated BB rats (▬) and control BB Wistar rats (——). Number of surviving animals in prophylactically insulin-treated group (----) and control group (----). ▨ Prophylactic insulin treatment period, days 1-93 (approximate age drawn as 50-142). (↓) First glucosuria test, day 95, in prophylactically insulin-treated animals

10 and 12 IU·kg^{-1}·day^{-1} for 16 days. In the second study, with similar animals, the doses used were 0, 12, 15 and 18 IU·kg^{-1}·day^{-1} for 21 days. Weight gain and hypoglycaemic reactions were registered.

Animals from eight litters of BB Wistar rats born within 5 days were randomly distributed into a control group (given the standard diabetes care) and a prophylactic insulin group (given heat-treated bovine ultralente insulin 15 IU·kg^{-1}·day^{-1}) from approximately 50 days of age (49.6 ± 1.5 days, SD $n = 36$) for a total of 93 days. Otherwise, the insulin group was treated the same as the control group. During the treatment period, blood samples were taken from the tail for blood glucose determinations (Reflocheck-Glucose, Boehringer-Mannheim, Mannheim, FRG) from animals showing weight loss or other signs of non-well-being.

Animals that died within the treatment period were autopsied, and samples of pancreas were fixed in Bouin's fixative. Paraffin sections were stained immunoenzymatically for A and B cells or with haematoxylin eosin for insulitis.

The group of prophylactic insulin-treated animals were tested for glucosuria 2 days after the 93rd injection. Diabetic animals were subsequently given individualized insulin doses according to the standard diabetes care procedures.

All non-diabetic animals in both the control and the prophylactic insulin group were given an intraperitoneal glucose tolerance test 2 weeks after the last prophylactic insulin injections. Tail vein blood samples were taken before, 1 h and 2 h after injection of 1.75 g glucose/kg. Blood glucose was determined on an autoanalyzer (Technicon Model IIc+, Swords County, Dublin, Ireland).

Results and Discussion

In normal Wistar rats, 15 IU·kg^{-1}·day^{-1} of the heat-treated bovine ultralente insulin was found to be a safe dose. In our ordinary BB Wistar colony, the daily dose of insulin to animals with diabetes of more than 2 months stabilized at 13 ± 3 IU·kg^{-1}·day^{-1} for male

rats and 20 ± 6 IU $\cdot$ kg^{-1} $\cdot$ day^{-1} for female rats. This was similar to what has been used to optimally control streptozotocin-induced diabetes in rats [7].

Figure 1 shows that a number of animals succumbed or were killed during the period of the study. The causes of deaths are summarized in Table 1. On day 57, all previously diagnosed diabetic animals in the control group showed extreme glucosuria and ketonuria and 2 female animals showed glucosuria for the first time. These animals and 3 female rats from the prophylactic insulin group had lost 23-57 g of weight during the preceeding weekend. Blood samples during the following period showed hyperglycaemia in these animals.

HPLC analyses of the remaining insulin in bottles used for injections the preceding 4 days showed that ordinary bovine ultralente insulin accidentally had been supplied to the animal house technician [6]. In spite of the reinstitution of injections with heat-treated bovine ultralente and close monitoring, the animals with ketonuria in both groups could not be saved.

From 2 of the 3 diabetic animals in the prophylactic insulin group, the pancreas could be processed for histology. Hematoxylin eosin stained sections showed typical insulitis and islet disorganization. Double immunoenzymatic staining showed highly irregular rims of A cells with rare B cells. That only female animals in the prophylactic insulin group died could be explained by the greater need for insulin among female diabetic animals in our BB rat colony.

An intraperitoneal glucose tolerance test (1.75 g/kg) on non-diabetic animals was performed two weeks after the last prophylactic insulin injection. Three of 20 control animals and 2 of 28 prophylactic insulin animals had blood glucose levels 1 and 2 h after the injection which exceeded mean values + 2 standards deviations for normal Wistar rats (1.66 mg/ml at 1 h, 1.36 mg/ml at 2 h) and the remaining non-diabetic BB Wistar rats.

The accumulated diabetes incidence in control animals and prophylactically insulin-treated animals is shown in Figure 1. Two days after the last prophylactic insulin injection, 6 of 36 animals in this group, 2 males and 4 females, had developed diabetes compared to 15 of 36 control animals, 7 males and 8 females ($p < 0.02$). One male control rat became diabetic on day 103 (aged 152 days, before the intraperitoneal glucose tolerance test), while one male and one female prophylactically insulin-treated rats became diabetic on day 122 and 136 (aged 171 and 185 days). The last 2 rats that developed diabetes 15 and 29 days after the intraperitoneal glucose tolerance test had normal glucose tolerance at the test.

At 230 days of age, when no further manifestations of diabetes were to the expected, a total of 8 of 36 prophylactically insulin-treated animals had developed diabetes compared to 16 of 36 control animals ($p < 0.05$).

The underlying mechanism for the reduction of the diabetes incidence by prophylactic insulin treatment has yet to be determined. Exogenous insulin would decrease the amount of endogenous pancreatic insulin synthesis and secretion. Insulinoma carrying rats had their pancreatic insulin content reduced by 90-95% [8]. B cells having a low rate of insulin synthesis and secretion could be less vulnerable to an autoimmune aggression; a similar protection phenomenon has been shown for cells concerning the cytotoxic action of streptozotocin [5]. Specifically, a reduction of endogenous insulin secretion might reduce the amount of antigen(s) on the B cell surface which are recognized by the immune system to such a level that the autoimmune cascade is either not initiated or alleviated.

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