DUAL AETIOLOGY DIABETES MELLITUS: A NOVEL MOUSE MODEL

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ABSTRACT

An experimental model of virus-induced diabetes has been developed in mice which are genetically not predisposed to pancreatic β cell damage due to coxsackievirus infection. Mice injected with subdiabetogenic doses of streptozotocin, a β cell toxin, became susceptible to diabetes upon infection with coxsackie B3. When the drug was administered after virus infection, this effect did not occur. The diabetic state caused by the two agents was permanent. These findings support the hypothesis that a combination of noxious agents and virus infection may result in diabetes mellitus.

INTRODUCTION

DIABETES mellitus has been reported in children following certain viral infections such as mumps, rubella and coxsackie B. The aetiological implication inherent in these clinical[1–5] observations has been supported by experimental diabetes in mice, induced by encephalomyocarditis virus[6,7]. Coxsackievirus infection also induces diabetes in certain genetically predisposed strains of mice such as CD 1, but not in others[8,9]. Therefore it has been suggested and supported by HLA studies[10] that genetic predisposition may be a factor in virus—induced diabetes mellitus in man.

Since diabetogenesis appears to be an uncommon result of coxsackievirus infection which is very common, we have proposed an alternative hypothesis that the convergence of the potentially diabetogenic infection and a toxin may possibly result in diabetes mellitus. In order to test this hypothesis, we had experimented and developed a mouse model of diabetes induced by a combination of a pancreatic β cell toxin and a virus infection, as reported previously[11]. In this paper, we present the experimental conditions necessary for the induction of diabetes.

MATERIALS AND METHODS

Swiss albino mice were obtained originally from Rockefeller Institute and a colony maintained locally for 15 years. Young male mice weighing 20-25 g were used in these experiments. Groups of 6 mice were inoculated intraperitoneally with 1000 $TCID_{50}$ of group B coxsackievirus, serotypes 1-6. Glucose tolerance was tested in these as well as control mice injected with medium alone, after 5, 12, 19 and 26 days. Animals were given a glucose load of 3 g/kg, after 16 h of fasting. Blood was collected from orbital sinus before and 60 and 120 min after glucose load and tested for reducing sugar[12,13]. The results were expressed as area under glucose tolerance curve (GTA) calculated as follows:

$$GTA = \frac{a+c}{2} + b$$

where a = fasting blood glucose (mg per 100 ml), b =blood glucose 60 min after load (ml)/100 and c = blood glucose 120 min after load, mg/100 ml). One month after inoculation, all mice in each group were bled, their sera pooled and neutralising antibody level to the inoculated agent determined.

Streptozotocin (SZN, Upjohn Company, Kalamazoo, USA) was dissolved in ice cold citrate buffer pH 4·5 just before use and was injected intraperitoneally into groups of 7 mice. Two dose levels namely 50 mg/kg or 75 mg/kg were used. SZN-treated mice were inoculated with 1000 $TCID_{50}$ of each of the six serotypes of group B coxsackievirus, after 1, 2, 7, and 15 days; control animals were given only SZN. GTA was done 5, 12, 26, 91 and 119 days after the virus inoculation in the former and after SZN injection in the latter groups. Diabetic mice were again tested 420 days after infection.

In a third experiment 1000 $TCID_{50}$ of coxsackie B3 (CB3) was inoculated in groups of 6 mice followed by SZN (75 mg/kg) injection on the same day or 1, 2, 3, 4, 6, 8 and 10 days later. In these animals GTA was done 5, 12 and 26 days after the injection of the drug.

RESULTS AND DISCUSSION

The GTA values and antibody levels of mice injected with virus are presented in Table I. Although there was immunological evidence that most serotypes of viruses infected the mice, they did not cause abnormal glucose tolerance in them. This strain of mice is therefore genetically not susceptible to diabetes mellitus caused by group B coxsackieviruses. CB3 appeared to be the most efficient infectious agent on account of the highest antibody response.

TABLE I

Glucose tolerance and immune response in mice inoculated with virus

Inoculated with	Mean GTA on post-inoculation day				Antibody titre to inoculated agent
	5	12	19	26	
Medium only (control)	221	215	ND	241	—
Coxsackie B1	232	243	232	255	8
Coxsackie B2	228	225	230	230	<4
Coxsackie B3	210	222	221	217	256
Coxsackie B4	220	224	219	220	16
Coxsackie B5	222	225	212	213	8
Coxsackie B6	226	215	220	219	8

ND = Not done

SZN is a toxin specific for the beta cells of pancreas[14]. Its effect is dose-dependent[15]. A dose of 50 mg/kg body weight does not cause any change in the glucose tolerance of mice whereas a dose of 75 mg/kg results in a mild diabetes, from which animals recover by about the 91st day[15]. Therefore we have chosen "sub-diabetogenic" dose levels of 50 and 75 mg/kg body weight in subsequent experiments.

Subdiabetogenic doses of the drug and coxsackievirus B1, 2, 4, 5 and 6 did not alter the glucose tolerance of the animals. However, the response to coxsackie B3 was distinctly different. The glucose tolerance of mice injected with 50 mg/kg of SZN and inoculated with CB3 is presented in Table II. Drug alone did not cause abnormal glucose tolerance. SZN and CB3 together produced only a slight abnormality in glucose tolerance, from which most groups of animals recovered by day 47 and others by day 68 or 91. In other words the addition of CB3 inoculation to 50 mg/kg dose of SZN resulted in changes similar to those produced by 75 mg/kg dose of SZN as discussed below.

The glucose tolerance following treatment with 75 mg/kg of SZN and virus is presented in Table III. While drug alone induced a slight hyperglycemia from which animals recovered by day 91, treatment with both agents induced a more pronounced hyperglycemia. The effect was maximal when virus was inoculated on the 2nd or 7th day after SZN. Figure 1 represents the glucose tolerance of mice inoculated with CB3 alone, with 75 mg/kg dose of the drug alone, and their combination at an interval of 7 days. The animals continued to be diabetic for 420 days, when the experiment was concluded.

The effect of virus infection prior to the administration of SZN on glucose tolerance of mice is presented in Table IV. This method resulted in only a mild diabetes, resembling the response to 75 mg/kg

TABLE II

Effect of streptozotocin (50 mg/kg) and CB3 infection on glucose tolerance of mice

Treated with	Interval between drug and virus (day)	Mean GTA ± SD on post-inoculation day					
		5	12	26	47	68	91
Coxsackie B3 only	—	252±4	264±9	246±10	ND	ND	ND
SZN (50 mg/kg) only	—	261±7	272±2	255±14	250±5	249±8	245±6
SZN and CB3	1	393±4	443±4	435±8	327±3	300±2	252±2
SZN and CB3	2	373±7	431±5	420±5	327±4	295±3	254±3
SZN and CB3	5	366±9	436±3	408±2	313±2	308±5	248±6
SZN and CB3	7	356±3	407±5	353±3	254±8	248±11	ND
SZN and CB3	15	310±3	336±4	336±4	250±2	241±5	ND

ND = Not done

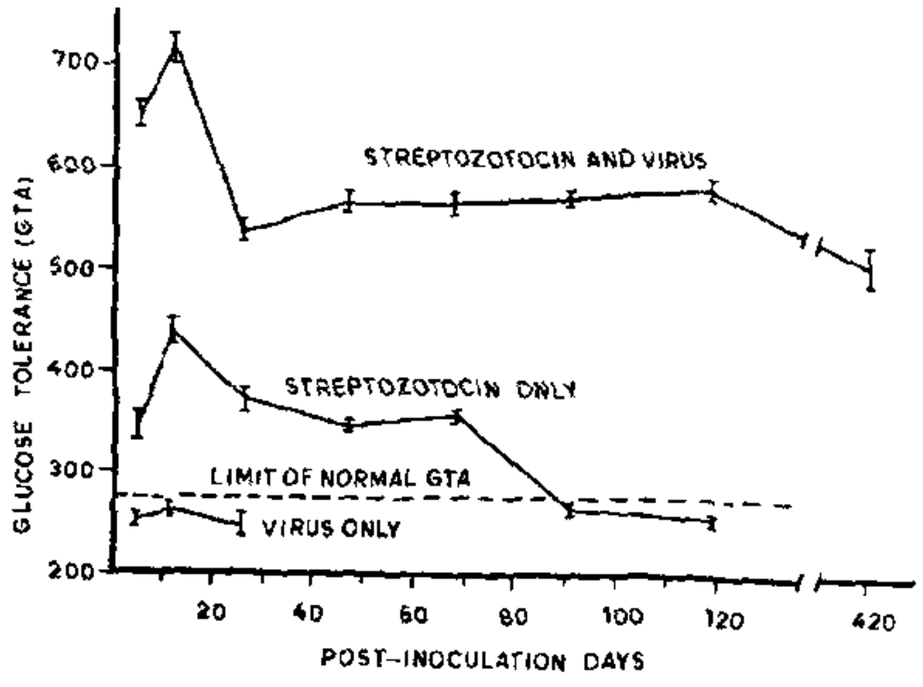

FIG. 1. Effect of CB3, SZN (75 mg/kg) and their combination on glucose tolerance in mice. Values presented as mean ± 1 SD.

dose of SZN alone. Thus virus infection prior to beta cell damage is ineffective in amplifying the hyperglycaemic state produced by a dose of 75 mg/kg of SZN.

These results show that there are interactions between the effects of the drug and of infection causing enhanced damage of β cells. The sequence of and the interval between the two stimuli are important in determining the severity of diabetes mellitus.

In human diabetes coxsackie B4 (CB4) has been shown to be the most frequently associated viral agent[2]. In experiments in randomly bred HAM/ICR strain of mice CB4, and in CDl strain of mice, CBl and CB4 were found to be diabetogenic[9,16]. More recently both CB3 and CB5 were found to induce diabetes in strains of mice normally susceptible to

TABLE III

Effect of streptozotocin (75 mg/kg) and CB 3 infection on glucose tolerance of mice

Treated with	Interval between SZN and CB3	Mean GTA ± SD on post-inoculation day							
		5	12	26	47	68	91	119	420
SZN (75 mg/kg only)	—	323 ± 8	456 ± 12	364 ± 5	350 ± 7	355 ± 4	261 ± 5	257 ± 3	ND
SZN and CB3	1	387 ± 12	564 ± 13	534 ± 13	539 ± 6	536 ± 6	522 ± 7	529 ± 6	ND
SZN and CB3	2	616 ± 12	673 ± 18	566 ± 22	582 ± 11	588 ± 6	594 ± 5	595 ± 6	ND
SZN and CB3	7	651 ± 19	719 ± 16	535 ± 9	569 ± 11	569 ± 6	572 ± 4	581 ± 2	509 ± 16
SZN and CB3	15	491 ± 4	648 ± 16	500 ± 11	517 ± 8	519 ± 4	509 ± 5	511 ± 4	ND

ND = Not done

TABLE IV

Effect of CB3 infection followed by streptozotocin injection on glucose tolerance of mice

Treated with	Interval between virus and SZN	Mean GTA ± SD on post-injection day		
		5	12	26
SZN (75 mg/kg only	—	346 ± 17	440 ± 18	368 ± 18
Virus and SZN	same day	433 ± 14	513 ± 12	340 ± 11
Virus and SZN	1	439 ± 8	528 ± 14	353 ± 17
Virus and SZN	2	434 ± 7	497 ± 7	392 ± 2
Virus and SZN	3	382 ± 17	445 ± 18	349 ± 11
Virus and SZN	4	334 ± 11	462 ± 12	380 ± 14
Virus and SZN	6	433 ± 18	510 ± 18	ND
Virus and SZN	8	349 ± 13	406 ± 14	ND
Virus and SZN	10	362 ± 10	403 ± 2	ND

ND = Not done

encephalomyocarditis (EMC) virus-induced diabetes, when they were pre-treated with sub-diabetogenic doses of SZN. EMC-M, a diabetogenic variant of EMC virus but not EMC-B, a non-diabetogenic variant was found to induce diabetes in diabetes-resistant strains of mice when they were pre-treated with SZN[17]. We had earlier demonstrated that among group B coxsackieviruses, only CB3 is capable of inducing chronic diabetes in mice which are genetically not predisposed to coxsackie-induced diabetes, after they were pre-treated with sub-diabetogenic doses of SZN[11].

Therefore our hypothesis that a partial damage of beta cells by noxious agents may predispose for virus-induced diabetes has been established. In mice this effect could be demonstrated upto 15 days after the noxious agent was applied. We suggest that a combination of some beta cell damaging toxin in our environment and infection with potentially diabetogenic viruses may interact in causing human diabetes mellitus.

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