ASSESSMENT OF THE ANTI-DIABETIC ACTIVITY OF DEOXYSPERGUALIN IN LOW-DOSE STREPTOZOTOCIN-INDUCED DIABETIC MICE

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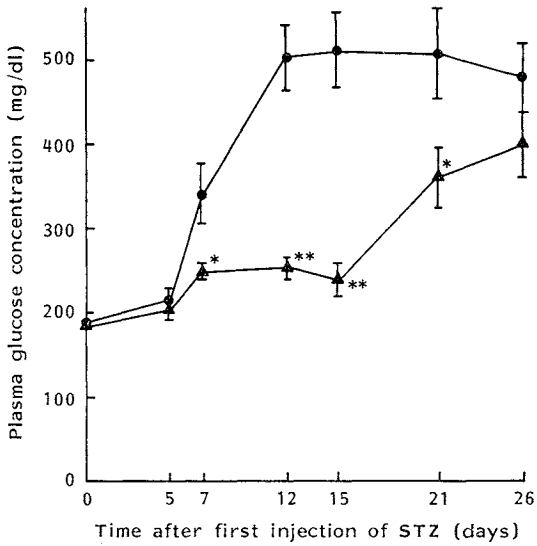
In mice, five injections of sub-diabetogenic doses of streptozotocin (STZ) may cause diabetes mellitus associated with lymphocyte infiltration of pancreatic islets[1,2]. There is evidence implicating autoimmune processes in the pathogenesis of this experimental diabetes[3,4]. Significantly, the development of this diabetes was prevented by rabbit anti-mouse lymphocyte serum[5]. Since deoxyspergualin (NKT-01) has strong immunosuppressive activities in rodents[6] and dogs[7], the use of this agent to prevent the diabetes induced by low-dose STZ has been investigated.

Six-week old male ICR mice (Clea Japan Co., Ltd., Tokyo, Japan) were used in all experiments. NKT-01 was supplied by Takara Shuzo Co., Ltd. It was dissolved in saline, sterilized by passing through 0.22 μm filter and stored at $-20°C$ until use. Cyclosporin A (Cy A, Sandimmun) was purchased from Sankyo Co., Ltd., Tokyo, Japan, and diluted with olive oil as required. STZ (Sigma, St. Louis, U.S.A.) was dissolved in 0.1 M sodium citrate buffer, pH 4.5, and used within 15 minutes after preparation. Blood samples were collected from the retro-orbital plexus using heparinized hematocrit capillary tubes. Plasma was quickly separated by centrifugation and assayed immediately. Plasma glucose was measured by a glucose oxidase method using a Rapid Blood Analyzer Super (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). Data were analyzed by Student's t-test.

Fig. 1. Changes of plasma glucose values in ICR mice received STZ alone (●) and STZ plus NKT-01 (▲), respectively.



STZ was ip injected at a sub-diabetogenic dose of 40 mg/kg once a day for 5 days. NKT-01 was ip administered at a daily dose of 6 mg/kg for 14 days from the initial day (day 1) of STZ injection. Each circle is shown as mean with SE from 10 mice. * $P<0.05$, ** $P<0.01$.

NKT-01 was used prophylactically to prevent the development of hyperglycaemia in mice treated with STZ (Fig. 1). Plasma glucose values in mice receiving STZ alone increased rapidly after the 5th injection of STZ and reached a plateau of about 500 mg/dl 12 days after the initial injection of STZ. When NKT-01 was ip administered at a daily dose of 6 mg/kg for 14 days after the 1st injection of STZ, the increase in the plasma glucose was significantly suppressed during the administration of NKT-01. However, cessation of the NKT-01 administration was followed by the marked development of hyperglycaemia.

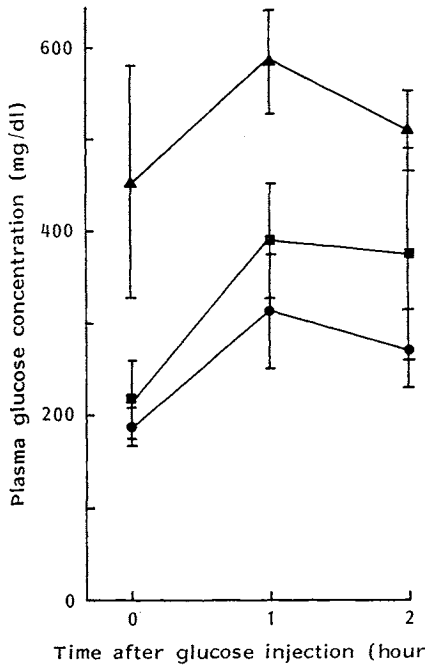
Results showing the dose dependency of NKT-01 and Cy A are summarized in Table 1. A significant prevention of the development of hyperglycaemia was observed at a daily oral dose of NKT-01 of 96 mg/kg or a daily ip dose of 6 mg/kg. These results reveal that oral and

Table 1. Effects of NKT-01 and Cy A on low-dose STZ-induced diabetes.

Group	Dose (mg/kg)	n	Administration route	Plasma glucose concentration (mg/dl)	Body weight (g)
Control		20		419±38	35.2±0.6
NKT-01	1.5	10	ip	428±49	36.3±0.5
	3	9	ip	430±49	35.8±0.8
	6	9	ip	250±27**	34.4±0.6
	24	10	po	439±34	33.8±0.6
	48	10	po	341±50	34.4±0.6
	96	10	po	250±32**	33.3±0.6
Cy A	25	8	po	577±40*	34.1±0.7
	50	10	po	568±28**	32.1±0.5

STZ was injected as described in the legend of Fig. 1. NKT-01 and Cy A were administered for 14 days once a day from the 1st day of STZ injection. The measurement of the body weight and the determination of plasma glucose were performed on the day of and after the 14th administration of each agent, respectively. Data are shown as mean with SE.
* $P<0.05$, ** $P<0.01$.

Fig. 2. Glucose-tolerance tests.



Plasma glucose response following ip injection of glucose (1 g/kg) in mice which received STZ alone (▲, $n=8$) and the combination of STZ and NKT-01 at a dose of 6 mg/kg (■, $n=7$) as described in Fig. 1. The test was performed 15 days after the 1st injection of STZ. Also shown are the plasma glucose values of 4 normal mice given ip with the same dose of glucose (●). Each symbol is shown as mean with SD.

Table 2. Effect of curative treatment of NKT-01 on low-dose STZ-induced diabetes.

Agent	n	Plasma glucose concentration (mg/dl)		
		Time after 1st injection of STZ		
		5 days	10 days	15 days
Control	10	236±12	392±36	452±24
NKT-01	10	257±14	378±28	429±23

STZ was injected as described in the legend of Fig. 1. NKT-01 was ip administered at a daily dose of 6 mg/kg for 10 days from the 5th injection of STZ. Data are shown as mean with SE.

ip efficacy is comparable within a dose ratio of about 16:1. The combination of STZ and NKT-01 did not have a toxic effect on these mice for body weight. In contrast, administration of Cy A at daily oral dose of 25 and 50 mg/kg led to significantly enhanced hyperglycaemia following STZ injections. In a preliminaly experiment, when Cy A was po administered at a daily dose of 100 mg/kg following the same schedule, 5 out of 10 mice died within the 5th day of STZ injection. Then, the remaining 5 mice had a plasma glucose of 333±73 mg/dl which was quite high.

As shown in Fig. 2, each pattern of the glucose tolerance curve for animals received STZ alone, and STZ plus NKT-01 (6 mg/kg) was similar to that of normal mice. Although the plasma glucose levels before the glucose injection in the mice administered NKT-01 was not statistically

different from that of normal mice, the NKT-01 administered mice had a different level at 1 hour ($P<0.05$) after the glucose injection compared to normal mice.

Finally, we examined the curative use of NKT-01 on diabetes. NKT-01 administered ip at a dose of 6 mg/kg daily for 10 days from the final injection of STZ did not prevent the occurrence of hyperglycaemia (Table 2).

The present study shows that NKT-01 given prophylactically can prevent the onset of diabetes induced by 5 low-dose injections of STZ. In contrast, NKT-01 failed to have a curative effect on STZ induced diabetes. NKT-01 was shown to have a stronger activity than Cy A in preventing the hyperglycaemia which follows STZ injections. Indeed, in this study, Cy A treatment was found to significantly enhance the hyperglycaemic state induced by STZ. This latter finding is consistent with another studies reporting a similar *in vivo* effect of Cy A[8,9]. Further investigations on the use of long term treatment with NKT-01 in spontaneously diabetic NOD mice are now being carried out.

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