Inhibition of Diabetes in Non-obese Diabetic Mice by Nicotinamide Treatment for 5 Weeks at the Early Age

To know the effects of nicotinamide (NCT) treatment for 5 weeks at the early age on insulitis and development of diabetes in non-obese diabetic (NOD) mice, this experiment was performed. Ten ICR (Institute of Cancer Research) and 15 female NOD mice at 4 weeks of age were used. Mice were assigned to ICR and NOD groups, and NOD mice were randomely divided to control, and NCT-treated groups. NCT was administered to mice orally as a solution and in a dose of 500 mg/kg body weight a day from the age of 4 to 8 weeks. Diabetes onset was 18 weeks of age in control group, and 22 weeks of age in NCT-treated group. Cumulative incidences of diabetes at 25 weeks of age in control and NCT-treated NOD mice were 63 and 29%, respectively. Insulitis occurred in all NOD mice. Incidence of insulitis in total islets was decreased by NCT treatment in diabetic NOD mice, but intensity of insulitis was not improved by NCT treatment. Blood glucose level was increased markedly, and plasma insulin level was decreased by diabetes development in NOD mice. Plasma triglycerides and total cholesterol levels were increased in diabetic mice than in non-diabetic mice. In conclusion, these results suggest that NCT treatment for 5 weeks at the early age in NOD mice inhibits development of diabetes and insulitis in diabetic NOD mice. *(JKMS 1997; 12 : 293~7)*

Key Words : *Nicotinamide, Insulitis, Insulin dependent diabetes mellitus, NOD mice*

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Received : January 14, 1997
Accepted : May 22, 1997

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* This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1995.

INTRODUCTION

Insulin dependent diabetes mellitus (IDDM) is a chronic autoimmune disease and its symptoms represent the end point of a preclinical slow process of immune destruction of insulin producing β-cells that leads to an absence of intrinsic insulin secretion (1). The destruction of islet β-cells may be caused by autoimmune mechanisms, viral infection (2, 3), chemicals (4), or introduction of transgenes into the β-cell, each in a setting of some genetic predisposition (5). Dietary and other environmental factors also contribute to the development of diabetes (6, 7).

IDDM is preceded by prediabetic phase, and advantage has been taken of this opportunity to introduce medications aimed at halting the autoimmune destruction of β-cells. The possible delineation of this prediabetic phase can lead to an early immunotherapeutic approach (8). Pancreatic β-cell destruction appears to be mediated by complement-fixing islet cell antibodies (ICA) or cytotoxic factors released by CD8 or NK cells, which, either directly, or via the generation of free oxygen radicals, induce DNA strand damage in β-cells (9). In this point, strategies preventing IDDM include the administration of immunosupressive agents and antioxidants. Other potential strategies include the induction of specific oral tolerance, insulin prophylaxis, immunoenhencement therapy, and dietary manipulation (10).

Recently, many reports (11~16) suggest that the antioxidant is beneficial for reducing IDDM. Nicotinamide (NCT) is noted for antioxidant, and a derivative of the B vitamin.

In order to know the effects of NCT treatment at the early age on insulitis and development of diabetes in NOD mice, this experiment was done.

MATERIALS AND METHODS

Animals

Fifteen NOD mice, which was originated from ICR (Institute of Cancer Research) mouse strain, purchased from Jackson Laboratory (Bar Harbor, ME, USA) were maintained in specific pathogen free room of the Animal Care Unit of Yeungnam University College of Medicine

as a animal model for IDDM, and 10 ICR mice from KIST (Taejon, Korea) were used for non-diabetic normal mice. The animals were fed by the regular foods and only female animals were used for the experiments.

Groups

Mice were divided to ICR (n=10) and NOD (n=15) groups, and NOD mice were randomely assigned to control (n=8) and NCT-treated (n=7) NOD groups.

NCT treatment

NCT (Sigma, USA) was administered to mice orally as a solution and in a dose of 500 mg/kg body weight daily from age of 4 to 8 weeks (17).

Assessment of Diabetes

To assess diabetes, mice were monitored twice a week for urine glucose since 15 weeks of age. Diabetes was diagnosed when mice were glycosuric for at least 2 consecutive times.

Sacrifice of Animals

The experiment was done following 7 hours fasting at 20 to 25 weeks of age in diabetic mice, and at 25 weeks of age in non-diabetic mice. Anesthesia was carried out with pentothal sodium (40mg/kg), and blood sampling was drawn from inferior vena cava. Then, mice were sacrificed with exsanguination, and then pancreatic tissue was excised immediately.

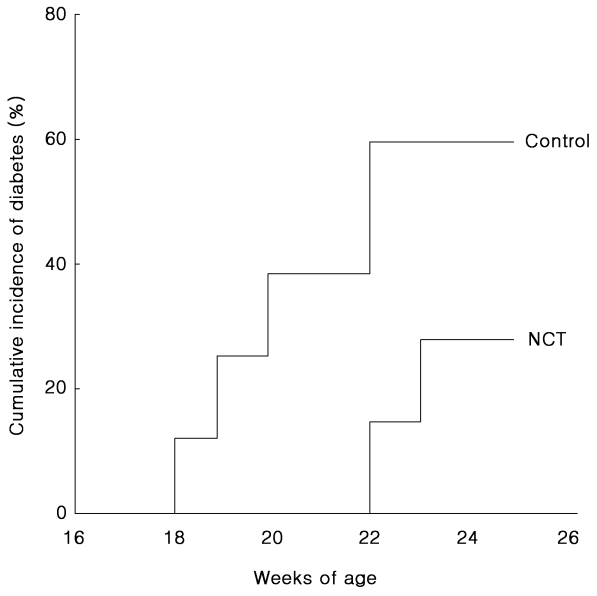

Fig. 1. Cumulative incidence of diabetes at 25 weeks of age in control and NCT-treated NOD mice.
NCT: nicotinamide.

Histology

Insulitis was assessed by histology at 20 to 25 weeks of age. Pancreatic tissue were prepared for histology by fixing in 10% neutral buffered formalin and then embedding in paraffin. The fixed blocks were sectioned to several non-adjacent sample and stained by hematoxylin and eosin. The samples were viewed by light microscopy.

Measurement of blood chemicals

Blood glucose was checked by glucose analyzer (Sidekick 1500, YSI, USA). Plasma insulin was measured by radioimmunoassay. Plasma triglycerides and total cholesterol were measured by enzyme method.

Statistical analysis

Statistical significances were determined by χ^2 test and one way ANOVA.

RESULTS

Diabetes onset was 18 weeks of age in control, but 22 weeks of age in NCT-treated NOD mice (Fig. 1). Cumulative incidences of diabetes was decreased (63% vs 29%, $p<0.05$) by NCT treatment in NOD mice (Table 1).

Insulitis was developed in all NOD mice, and incidences of total islets were decreased significantly ($p<0.05$) by NCT treatment in diabetic, but not in non-diabetic NOD mice. Intensity of insulitis was not change by NCT treatment (Table 2).

Blood glucose level was significantly ($p<0.001$) in-

Table 1. Cumulative incidence of diabetes at 25 weeks of age in ICR, control and NCT-treated NOD mice

Group	Diabetes	
	number	%
ICR, n=10	0	0
NOD		
Control, n=8	5	63
NCT, n=7	2	29

N indicates number of cases.
NCT: nicotinamide. NOD: non-obese diabetic.

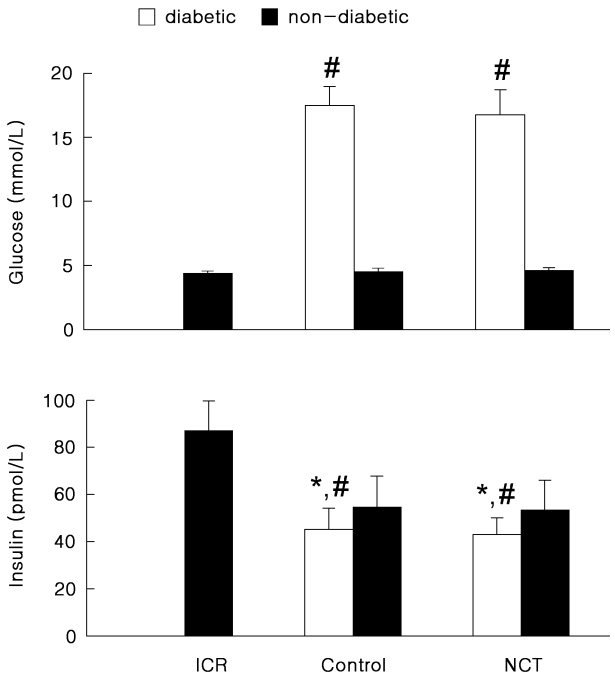

Fig. 2. Levels of blood glucose and plasma insulin in ICR, control and NCT-treated NOD mice. Values are mean±S.D.. NCT : nicotinamide. * $p<0.05$ vs diabetic. # $p<0.001$ vs non-diabetic mice in blood glucose level or vs ICR mice in plasma insulin level.

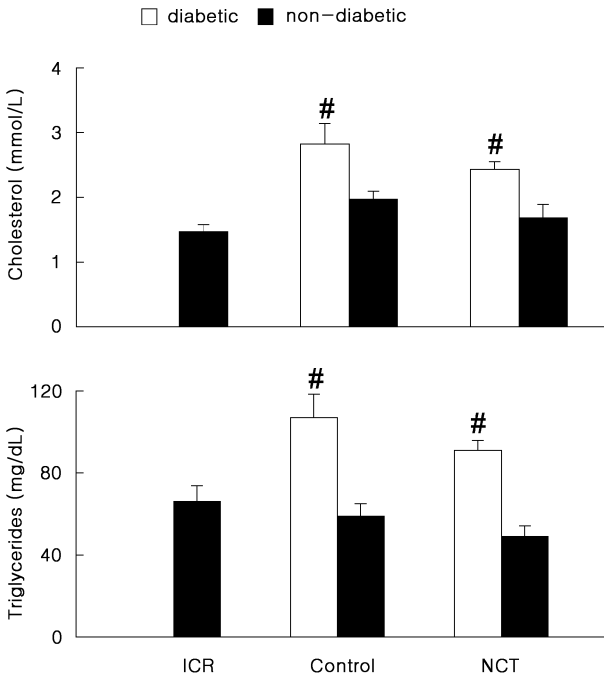

Fig. 3. Levels of plasma triglycerides and total cholesterol in ICR, control and NCT-treated NOD mice. Values are mean±S.D.. NCT : nicotinamide. # $p<0.001$ vs non-diabetic or ICR.

creased 3.6 to 3.9 fold by diabetes development in NOD mice. Plasma insulin level was lowered significantly ($p<0.001$) in NOD mice than in ICR mice, and decreased significantly ($p<0.05$) by diabetes development in NOD mice (Fig. 2).

Plasma triglycerides and total cholesterol levels were significantly ($p<0.001$) increased in diabetic mice than in non-diabetic mice (Fig. 3).

DISCUSSION

NOD mice is animal model of IDDM and is characterized by destruction of islet β-cells as consequence of autoimmune reaction.

In female NOD mice, insulitis occurs about the age of 5 weeks and the onset of glycosuria occurs around the age of 120 days (18). The frequency of diabetes in the

Table 2. Comparison of insulitis at 25 weeks of age among ICR, control and NCT-treated mice

Group	N	Number of islets counted	Insulitis(%)		Intensity of insulitis(%)		
			without	with	+	++	+++
ICR	10	41	100	0			
NOD							
Control							
diabetic	5	42	17	83	40	24	19
non-diabetic	3	31	42	58	32	19	7
NCT							
diabetic	2	19	27	73	38	24	11
non-diabetic	5	37	47	53	32	16	5

N indicates number of cases. NCT : nicotinamide. NOD : non-obese diabetic.
Intensity of insulitis are expressed as follows : + means islets with mild surrounding lymphocytes. ++ means islets with a few intra-islets and/or moderate to severe surrounding lymphocytes, and +++ means islets with many intra-islets and severe surrounding lymphocytes.

NOD mouse is sex-dependent, with approximately 90% of females and 40% of males developing clinical diabetes by 40 weeks of age (19). One potential mechanism for β-cell destruction is the toxic effect of free oxygen radicals produced as a result of the influx of inflammatory cells into the pancreas (19) and the release of cytokines (16, 20). Regarding mechanisms of cytokine-induced DNA damage, inhibition of nitric oxide generation by an arginine analogue, l-NMMA was reported to prevent interleukin-1β-induced DNA damage in rat islet cells and a hamster insulinoma cell line, identifying nitric oxide as a mediator of cytokine induced DNA damage (21). These results suggest that antioxidants may have therapeutic application in attempts to prevent immune- mediated islet β-cell damage and IDDM (22). In recent, some researchers reported a various antioxidant including NCT (12, 15), vitamin E (11), and probucol (14) prevent or inhibit the development of insulitis and diabetes in NOD mice. NCT not only is a free radical scavenger, but also inhibits interleukin-1β-induced nitric oxide synthase (23) and poly (ADP-ribose) synthetase (24) in islets.

In this experiment, diabetes developed at 18 weeks of age in control group, and at 22 weeks of age in NCT-treated NOD group. Cumulative incidences of diabetes at 25 weeks of age in control and NCT-treated NOD mice were 63 and 29%, respectively. Insulitis occurred in all NOD mice. Incidences of insulitis in total islets were decreased in diabetic NOD mice, but intensity of insulitis was not improved by NCT treatment in NOD mice.

Beta cell viability depends on NAD for maintaining NADP redox cycles, for DNA repair processes and for insulin synthesis. As a NAD precursor, NCT can be used to improve intracellular NAD content and ameliorate β-cell viability (25). In experimental models, such as the NOD mouse, the partially depancreatised rat and mice, and streptozotocin-induced diabetes, NCT has been shown to improve β-cell regeneration and, if administered before the onset of the disease, to prevent it. NCT in vitro is able to preserve β-cell function following the addiction of ICA (26). NCT protects isolated islets in vitro from the toxicity of a number of agents, but only in doses that produce significant PARP inhibition, and increased intracellular levels of NAD. Other effects of NCT are more likely, e.g., increase in NAD pool size by de novo synthesis, or inhibition of free radical generation (12). Therefore, NCT may prevent or inhibit destruction of β-cells from free radicals produced by cytokines and inflammatory cells.

In conclusion, our results suggest that NCT treatment for 5 weeks at the early age inhibits development of diabetes in NOD mice and insulitis in diabetic NOD mice.

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