Attempt to Prevent the Development of Diabetes in Non-Obese Diabetic Mice by Oral Vanadate Administration

Joseph Meyerovitch MD[1], Trevor Waner BVSc PhD[4], Joseph Sack MD[1], Juri Kopolovic MD[2] and Joshua Shemer MD[3]

[1]Pediatric Division, and Departments of [2]Pathology and [3]Internal Medicine F, Sheba Medical Center, Tel-Hashomer, and Sackler Faculty of Medicine, Tel Aviv University, and [4]Israel Institute for Biological Research, Ness Ziona, Israel

Key words: vanadate, non-obese diabetic mice, prophylaxis, protection, islet rest

Abstract

Background: Despite current treatment protocols, the long-term complications of insulin-dependent diabetes mellitus have prompted the investigation of strategies for the prevention of IDDM.

Objectives: To investigate the effect of oral vanadate in reducing diabetes type I in non-obese diabetic mice.

Methods: Sodium metavanadate, 3.92 mmol/L, was added to the drinking water of 8-week-old female NOD mice. Blood glucose levels, water consumption and body weight were measured, and the end point of the study was judged by the appearance of hyperglycemia in the mice.

Results: Treatment with vanadate did not significantly reduce the incidence of type I diabetes as compared to the control group. However, oral vanadate therapy significantly reduced the blood glucose levels after the fourth week of treatment compared to the control group (3.83±0.67 vs. 4.44±0.83 mmol/L, $P<0.03$). There was a consistent and significant increase in body weight of the vanadate-treated pre-diabetic NOD mice compared to the controls. Diabetic mice treated with vanadate had significantly lower levels of serum insulin as compared to control diabetic mice (104±27 vs. 151±36 μmol/L, $P<0.03$). Histologically, no significant differences were found in inflammatory response of the islets of Langerhans between the control and treated groups.

Conclusions: This study suggests that the post-receptor insulin-like effect induced by vanadate is not sufficient to prevent the development of diabetes and insulitis in pre-diabetic NOD mice.

IMAJ 2000;2:211–214

Insulin-dependent diabetes mellitus is an organ-specific autoimmune disease that involves the destruction of insulin-producing cells of the islets of Langerhans [1]. Despite current treatment protocols, the long-term microvascular and macrovascular complications of IDDM prompted the investigation of strategies for the prevention of IDDM [2]. The non-obese diabetic mouse has been demonstrated to be an excellent animal model of human IDDM [3].

Studies have shown that the administration of insulin to the NOD mouse and BB Wistar rat significantly reduces the incidence of diabetes [4,5]. Vanadate, a trace element, was found to be a potent insulinomimetic agent in isolated adipocytes [6], in streptozotocin diabetic rats [7–9], in rodent models of non-insulin-dependent diabetes [10], in cats [11], and in obesity [12]. Vanadate has been demonstrated to act distally to the tyrosine phosphorylation of its major substrate IRS1, thus supporting a post-insulin receptor mechanism of action [10,13].

In view of the success in reducing the incidence of diabetes in NOD mice by prophylactic insulin therapy, we undertook to investigate the prophylactic effect of oral administration of vanadate on pre-diabetic NOD mice. The aim of this study was to further elucidate the mechanism of prevention of diabetes mellitus by using a post-receptor mimicker of insulin.

Materials and Methods

Animals

Thirty female NOD mice aged 6 weeks were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and kept in groups of five in grid-bottomed polypropylene cages. The animals were housed in a barriered rodent facility, with room target temperature and humidity ranges set at 21±2°C and 55±15%, respectively. A commercially available laboratory animal diet was fed *ad libitum* throughout the study. All the mice were cared for according to the Guidelines for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, 1985).

Before experimentation, the mice were acclimatized for 2 weeks during which their health status was assessed daily by observation. Before commencement of the study the mice were weighed and their blood glucose levels measured using the "Glucometer Elite" (Bayer's Diagnostics, USA).

Experimental design

The animals were allocated to two groups using a random

IDDM = insulin-dependent diabetes mellitus
NOD = non-obese diabetic

number table. Group 1 (n=15) served as the control, and group 2 (n=15) received vanadate sodium (sodium metavanadate, BDH Chemicals Ltd., Poole, UK) in their drinking water at a concentration of 3.92 mmol/L. NaCl, 80 mmol, was added to the drinking water of both groups. Vanadate solutions were replaced every 3 days. The animals were inspected daily for signs of ill health. Each mouse was weighed once a week. Water consumption was calculated weekly, based on measurements carried out three times a week.

Blood glucose was measured once a week by nicking the tail and drawing a drop of blood. Mice with blood glucose levels of 11 mmol/L for 2 consecutive weeks were regarded as diabetic; they were anesthetized by ether inhalation, exanginated and sacrificed. A full necropsy was carried out on each mouse and selected organs were preserved in formalin.

Vanadate and insulin serum assays

Terminal blood samples were assayed for levels of vanadate by flameless atomic absorption spectroscopy using a Perkin-Elmer spectrophotometer (USA) [14]. The lowest limit of detection of vanadium was 0.98 μmol/L. Each sample was assayed in triplicate, from which a mean and standard deviation were derived. Serum samples were also assayed for insulin using a radioimmunoassay technique [15].

Histology

Formalin-fixed pancreas tissue was paraffin embedded, sectioned at 5 μm, stained with hematoxylin eosin and examined microscopically.

Data analysis

Differences in body weight gain, water consumption, blood glucose and serum insulin concentrations at different periods throughout the study were compared using the Student's *t*-test. Comparison of diabetes onset curves was carried out using the log rank test. Onset fractions were calculated using the Kaplan-Meier method. The median onset time for each group was calculated.

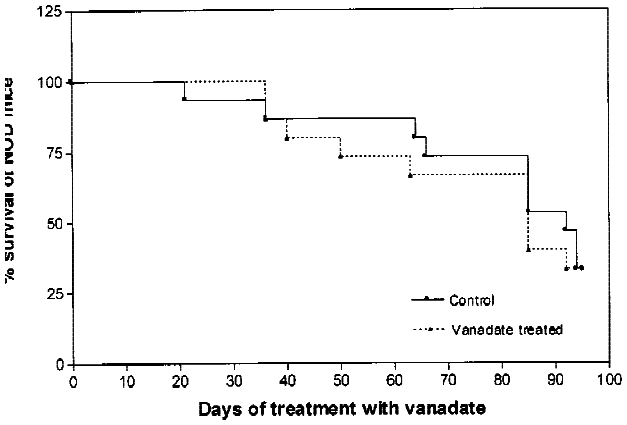


Figure 1. Incidence of diabetes mellitus in NOD mice receiving 3.92 mmol/L of sodium metavanadate in their drinking water (dotted line), compared to untreated NOD mice (solid line).

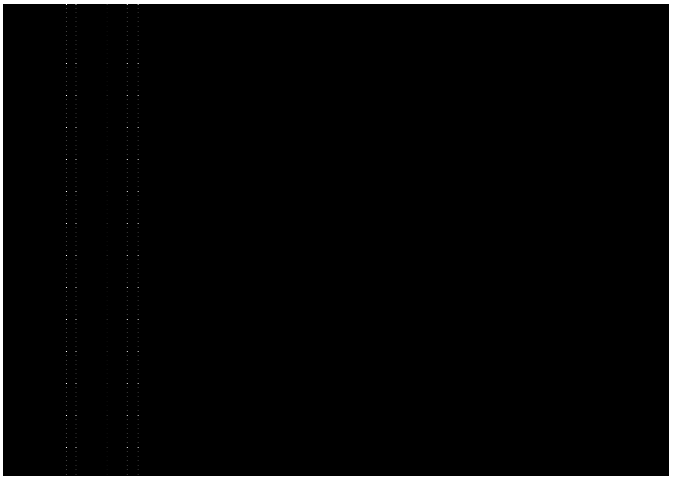


Figure 2. Mean (SEM) weekly change in body weight (g/week) of NOD mice. Solid line represents control mice and dotted line represents mice receiving 3.92 mmol/L of sodium metavanadate in their drinking water.

Results

Development of diabetes and survival rate

A graphic representation of the onset rate of diabetes in both groups of NOD mice during the experimental period is presented in Figure 1. The rate of onset of diabetes was similar for both the control and vanadate-treated groups ($P=0.7736$). By 94 days after initiation of treatment with vanadate, 67% of both groups had developed diabetes. The median survival time for control NOD mice was 92 days compared to 85 days for the vanadate-treated mice.

Water consumption

Pre-diabetic NOD mice given vanadate in their drinking water consumed on average 34% less water than the control NOD mice, which drank water containing 80 mmol/L NaCl.

Body weight

The changes in body weight for control and vanadate-treated NOD mice throughout the experimental period are shown in Figure 2. NOD mice given vanadate gained body weight at a significantly higher rate than the control group throughout the study period [Figure 2]. At age 23 weeks the control mice had gained a mean body weight of 5.7±0.6 g compared to 7.6±0.5 g in the vanadate-treated mice ($P<0.001$).

Blood glucose levels

Blood glucose levels were similar for both pre-diabetic NOD mice groups throughout the study, except for the period between weeks 4 and 7 after the treatment began, during which the vanadate-treated group displayed blood glucose values lower than those of the control group [Figure 3]. After 4 weeks of treatment a statistically significant difference was found for blood glucose concentration between the two groups (3.83±0.67 vs. 4.44±0.83 mmol/L, $P<0.03$).

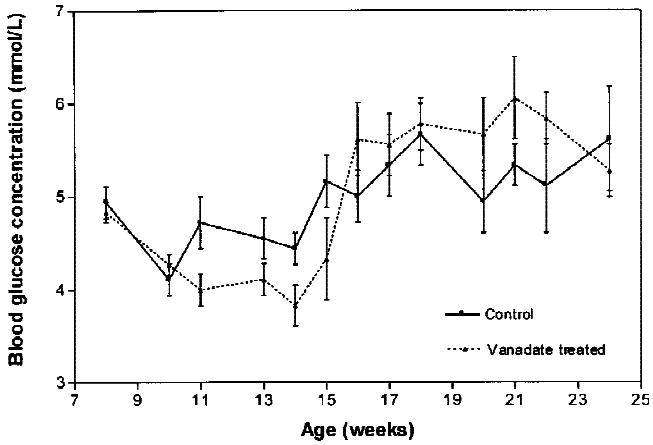


Figure 3. Mean (SEM) concentration of blood glucose (mmol/L) of NOD mice receiving 3.92 mmol/L of sodium metavanadate in their drinking water (dotted line), compared to untreated NOD mice (solid line). Note the reduced blood glucose levels for the vanadate-treated mice from week 4 to 7 after the initiation of treatment.

Serum insulin concentrations

Serum insulin levels in vanadate-treated diabetic NOD mice were significantly lower than those of the untreated diabetic NOD controls (104±27 vs. 151±36 μmol/L, $P<0.03$) at the terminal bleed.

Serum vanadium levels

Analysis of vanadium in the serum revealed values below the minimum level of detection in the control mice, compared to 19.6±3.9 mmol/L in diabetic mice receiving vanadate.

Histological assessment of the pancreas

Histological examination of the pancreas revealed no differences in the inflammatory response to the islets of Langerhans between the two groups.

Discussion

The oral administration of a therapeutic dose of sodium metavanadate to 8-week-old pre-diabetic NOD mice for 18 weeks resulted in an initial decline in blood glucose levels and an increase in body weight gain throughout the study. However, vanadate treatment did not reduce the incidence of diabetes in pre-diabetic NOD mice. In contrast, this same treatment protocol was effective in correcting hyperglycemia and other metabolic defects associated with diabetes of streptozotocin diabetic rats [8], and in two mice NIDDM models: ob/ob and db/db [10]. Serum vanadate levels in the treated NOD mice were 19.6 mmol/L, similar to those reported to be effective in causing normoglycemia in SZT rats and ob/ob mice [8,10].

The mechanism of action of insulin in the prevention of diabetes is unclear, although the hypothesis of "islet rest" has been put forward to explain this beneficial effect [4,5]. According to this hypothesis, the pancreatic cells express reduced quantities of putative autoantigens, thus diminishing the autoimmune process. Treatment with vanadate has been reported to supplement insulin deficiency and suppress insulin secretion [16,17]. In the present study we also demonstrated significantly lower levels of insulin in the diabetic NOD mice treated with vanadate, as compared to the hyperglycemic NOD mice that did not receive any treatment.

There are several explanations for the inability of the vanadate treatment to prevent diabetes mellitus in NOD mice. The insulin treatment, which was effective in preventing IDDM in NOD mice and BB Wistar rats, may have caused a more profound hypoglycemic effect compared to that induced by vanadate. Since blood glucose concentrations were not reported in either of the above studies it is not possible to compare our results.

The effectiveness of insulin prophylaxis in NOD mice may have been due to the presence of high titers of insulin antibodies produced by the immunization scheme, resulting in the blocking of the autoimmune process. Alternatively, the repeated injection of foreign protein (namely, pork insulin) may have altered a defective T lymphocyte function, thus reducing the incidence of IDDM in NOD mice and BB rats. Treatment of NOD mice and humans with complete Freund's adjuvant significantly reduced the incidence of diabetes, possibly by altering the nature of the destructive process of the islets of Langerhans [18]. Furthermore, the administration of a peptide p277 derived from 60 kDA heat shock protein (hap60) also arrested the autoimmune process, even at advanced stages [19]. Recently Muir and colleagues [20] reported that intermittent immunizations with insulin or its inactive β-chain in incomplete Freund's adjuvant prevented diabetes in NOD mice by diminished intra-islet interferon-γ transcription.

In the present study, serum vanadate levels (about 20 mmol/L) were similar to those reported in the treatment of hyperglycemia in SZT-treated rats [8,10]. At this concentration a long lasting normoglycemia, and a shift from a catabolic to anabolic state, was demonstrated in the STZ-treated rats. In the present study vanadate treatment did cause a temporary decline in blood glucose concentration in the NOD mice from week 4 to 7 after initiation of treatment. The anabolic effect of vanadate on the NOD mice was demonstrated by the significant increase in body weight in the vanadate-treated mice as compared to control animals. It is possible that strain differences may be responsible for the differential effect of normoglycemic versus the anabolic state.

In conclusion, we report that the post-receptor insulinomimetic action of vanadate did not reduce the incidence of diabetes in NOD mice. Signs of vanadate's insulinomimetic effect in this study included a temporary decline in blood glucose levels, an increase in body weight, and a decrease in serum insulin concentrations. The results suggest that the immunological effects of insulin as an antigen may be more

SZT = streptozotocin

important in conferring its prophylactic properties, as demonstrated by the lack of effect of vanadate as an insulinomimetic with a post-insulin receptor action. These results may have a bearing on current prophylactic protocols using insulin in pre-diabetic human patients [21].

Acknowledgments: We wish to acknowledge the kind assistance and skillful analysis of serum vanadium concentrations by Dr. H. Barak and Dr. I. Chamham.

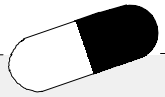
References

1. Castano L, Eisenbarth GC. Type 1 diabetes: a chronic auto-immune disease of human, mouse and rat. *Annu Rev Immunol* 1990;8:647–79.
2. Robertson RP, Kiein DJ. Treatment of diabetes mellitus. *Diabetologia* 1992;35(Suppl 2):S8–17.
3. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochini Y. Breeding of nonobese diabetic strain of mice. *Exp Anim* 1980;29:1–13.
4. Gotfredsen CF, Buschard K, Fradsen EK. Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. *Diabetologia* 1985;28:933–5.
5. Atkinson MA, Maclaren NK, Luchetta R. Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. *Diabetes* 1990;39:933–7.
6. Green A. The insulin-like effect of sodium vanadate on adipocyte glucose transport is mediated at post-insulin receptor level. *Biochem J* 1986;238:663–9.
7. Heyliger CE, Tahiliani A, Mcneill TH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science* 1985;227:1474–6.
8. Meyerovitch J, Farfel Z, Sack J, Shechter Y. Oral administration of vanadate normalized blood glucose levels in streptozotocin-treated rats. *J Biol Chem* 1987;263:6658–62.
9. Shechter Y, Meyerovitch J, Amir S. The use of post-receptor agent in studying insulin action and relation to experimental diabetes. *Biochem Pharmacol* 1988;37:1891–6.
10. Meyerovitch J, Rotenberg P, Shechter Y, Bonner Y, Weir S, Kahn CR. Vanadate normalizes hyperglycemia in two mouse models of non-insulin dependent diabetes mellitus. *J Clin Invest* 1991;87:1286–94.
11. Plotnick AN, Greso DS, Crans DC, Elfrey S. Oral vanadium compounds: preliminary studies on toxicity in normal cats and hypoglycemic potential in diabetic cats. Proceedings of the 13th Veterinary Medical Forum, American College of Veterinary Internal Medicine. *J Vet Int Med* 1995;9:181.
12. Brichard SM, Pottier AM, Henguin JC. Long term improvement of glucose homeostasis by vanadate in obese hyperinsulinemia fa/fa rats. *Endocrinology* 1989;125:2510–16.
13. Shisheva A, Shechter Y. Role of cytosolic tyrosine kinase in mediating insulin-like action of vanadate in rat adipocytes. *J Biol Chem* 1994;268:6463–9.
14. Stroop SD, Helinek G, Green E. More sensitive flameless atomic absorption analysis of vanadium in tissue and serum. *Clin Chem* 1982;28:79–82.
15. Bates HM. Insulinoma and pheochromocytoma. *Lab Management* 1983;15:11–12.
16. Malabu UH, Dryden S, Mccarthy HD, Kilpatric A, Williams G. Effect of chronic vanadate administration in the STZ-induced diabetic rat. The hyperglycemic action of vanadate is attributable entirely to suppression of feeding. *Diabetes* 1994;43:9–15.
17. Mcinerey MF, Pek SB, Thomas DW. Prevention of insulitis and diabetes onset by treatment with complete Freund's adjuvant in NOD mice. *Diabetes* 1991;40:715–25.
18. Shehadeh N, Calcinaro F, Bradley BJ, Bruchlim I, Vardy P, Lafferty KJ. Effect of adjuvant therapy on development of diabetes in mouse and man. *Lancet* 1994;343:706–7.
19. Elias D, Cohen IR. Peptide therapy for diabetes in NOD mice. *Lancet* 1994;343:704–6.
20. Muir A, Peck A, Clare-Salzier M, Yao-Hua S, Cornelius J, Luchetta R, Krischer J, Maclaren N. Insulin immunization of non-obese diabetic mice induces a protective insulitis characterized by diminished intraislet interferon-γ transcription. *J Clin Invest* 1995;95:628–34.
21. Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type 1 diabetes. *Lancet* 1993;341:927–8.

Correspondence: Dr. T. Waner, Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 74048, Israel. Tel: (972-8) 938 1461; Fax: (972-8) 940 1240; email: wanertnt@shani.net

If you want the rainbow you got to put up with the rain.
Dolly Parton

Capsule



A class difference in MHC

The adaptive immune response is stimulated when peptides that are bound to major histocompatibility complex (MHC) molecules bind to T cells. Structures of T cell receptors (TCRs) in complex with peptide-MHC class I ligands have been determined. Reinherz et al. present the structure of a TCR binding to a complex of a 16-residue peptide antigen and a murine MHC class II molecule (pMHCII). The authors propose that the angle of TCR-pMHCII docking will be restricted to a more "orthogonal" interaction than that seen in the class I "diagonal" mode, and that this difference in docking specifies the preferential binding of pMHCI and pMHCII to CD8 or CD4 co-receptors, respectively. In a perspective, Wilson analyzes the similarities and differences between the pMHCII and pMHCI structures, and cautions that while this work is a valuable contribution, more TCR-pMHC structures are needed to understand fully the structural basis of TCRMHC recognition.

Science 1999;286:1913