J. Cell. Mol. Med. Vol 7, No 4, 2003 pp. 447-454

The influence of sodium metavanadate on the process of diabetogenesis in BB rats

D. Cheta [a] *, Gabriela Orasanu [a], T. Nicolaie [b], Dana Iordachescu [c], S. Buligescu [a], C. Constantin [b], M. Hassanain [a], Anca Coman [a], Mihaela Enache [c], Raluca Negru [a], Valeria Tica [c], Delia Timofte [b], Daniela Gutu [a], C. Panaite [d]

[a] *"N. Paulescu" Institute, 2nd Clinic of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania*
[b] *Central Military Hospital, 1st Medical Clinic, Bucharest, Romania*
[c] *Department of Biochemistry, University of Bucharest, Bucharest, Romania*
[d] *"Carol Davila" University of Medicine and Pharmacy, General Medicine Faculty, Bucharest, Romania*

Received: February 7, 2003; Accepted in revised form: November 30, 2003

Abstract

Vanadium has been shown to be beneficial in the oral treatment of animal models of type 1 and type 2 diabetes. The aim of the study was to evaluate the short-term effects of sodium metavanadate in prediabetic BB-DP rats. To do this, 96 rats were divided into 4 equal groups. Groups V1, V2, V3 were treated with sodium metavanadate (0.1, 0.2 and 0.3 mg/ml respectively) and sodium chloride (0.5 mg/ml) in drinking water for 7 days. Group C received only sodium chloride (0.5 mg/ml). Blood glucose (BG), glycosuria, ketonuria, body weight and insulinemia were determined. The age of onset of diabetes was significantly higher for groups V2, V3 compared to group C, ($p<0.05$) and depends on the metavanadate concentration (V3 vs. V1, $p=0.006$). The incidence of diabetes was lower in the rats treated with metavanadate than in the control group, but this difference was not statistically significant. In diabetic rats, the BG at the onset was higher in group C than in groups V, $p<0.05$. Insulinemia, at the onset of the treatment as well as immediately after its cessation showed a drop in the treatment groups, proportionally to the dosage of vanadium, but later increased slowly and continuously until the end of the experiment. In conclusion, metavanadate delays the development of diabetes in BB-DP rats, but does not prevent its onset. A milder form of diabetes occurs in diabetic rats treated with metavanadate. The effects depend on the metavanadate concentration and 0.2 mg/ml is preferable.

Keywords: type 1 diabetes - sodium metavanadate - BB-DP rats - insulin-mimetic - prevention - beta-cells

Introduction

The prevention of diabetes mellitus is an urgent necessity for medicine and the human society at the beginning of this millennium. Epidemic growth, devastating complications, huge costs and other arguments support the above assertion [1]. Prevention projects for type 1 diabetes have a strong experimental basis. There is an impressive array of methods for prevention of type 1 diabetes in animal models [2].

Insulin replacement is the easiest method of controlling diabetes. There is great interest in orally

* Correspondence to: Dan CHETA, MD, PhD
28, Alexandru Donici Street,
020479 Bucharest 2, Romania.
Tel.: (4021) 211-8514, Fax: (4021) 211-1575
E-mail: fpas@fx.ro

active insulin-mimetics, particularly vanadium compounds [3]. Because vanadium salts are also readily soluble in water and rapidly absorbed into the bloodstream following oral administration, it was of interest to determine if vanadium *per os* would be beneficial in the prevention and treatment of diabetes [4].

Vanadium compounds exert a variety of biological effects, the most notable being their effects as insulin-mimetics [5]. They effectively control the diabetic state and prevent the development of complications in both insulin-dependent and non-insulin-dependent diabetes mellitus in experimental animals [6]. Clinical studies in type 1 diabetes patients showed that the daily insulin requirement decreased significantly [7]. In type 2 diabetic patients, administration of vanadium determined an increase of insulin sensitivity partly by enhancing the inhibiting effect of insulin on hepatic glucose production, and stimulating peripheral glucose uptake [7]. A vanadium-induced increase in GLUT4 glucose transporter levels was observed in diabetic skeletal and cardiac rat muscle [8–10].

It has been clearly established that vanadium compounds are potent phosphotyrosine phosphatase (PTP) inhibitors. The insulin receptor kinase (IRK) is intimately associated with one or more PTPs especially during the course of its intracellular itinerary in the endosomal system of the cell [11]. Inhibition of the IRK-associated PTPs by vanadium allows IRK auto-phosphorylation and activation in the complete absence of insulin, followed by all the known insulin responses [11]. While the precise biochemical pathway of vanadate action is not completely known, it has the ability to "by-pass" defects in insulin action in diseases characterized by insulin resistance [7].

A remarkable property of vanadium is that it can reverse the diabetic state for a long time after withdrawal from treatment [12]. Vanadium preserves beta-cells in streptozotocin (STZ)-induced diabetes at least partially by abolishing the insulin hypersecretory response followed by exhaustion of residual insulin stores after a moderate dose of STZ [12]. Vanadyl or vanadate long-term islet-protective effects are evident if treatment is initiated before islet destruction has reached a critical level. Several reports have demonstrated that vanadate also mimics the late effects of insulin related to mitogenesis, by activating the MAP (mitogen activated protein)-kinase cascade [13].

Our previous studies and some other reports [14] have shown that chronic prophylactic exogenous insulin treatment commenced prevented type 1 diabetes in young diabetes susceptible BB rats. These results support the hypothesis that decreased beta-cell activity is responsible for the protection against the immune beta-cell destruction [14].

In this study, we have attempted to evaluate the presumed preventive effect of sodium vanadate in prediabetic BB-DP rats, which spontaneously develop autoimmune diabetes mellitus, closely resembling human type 1 diabetes.

Materials and methods

Animals

We used 96 Bio-Breeding Diabetes Prone (BB-DP) rats (140-250g) of prediabetogenic age (40-50 days), obtained from Dr. P. Thibert ("Sir Frederick Banting Research Center", Ottawa, Canada). They were bred in specific pathogen free conditions at "I. Cantacuzino" Institute, Bãneasa Station, Romania.

Treatment and maintenance

The rats were divided into 4 equal groups (considering sex, age and weight). Groups V1, V2, V3 (24 rats per group) were treated with different doses of sodium metavanadate (0.1, 0.2 and 0.3 mg/ml respectively) and sodium chloride (0.5 mg/ml) in drinking water for 7 days. Group C (24 rats), the untreated control group, received only sodium chloride (0.5 mg/ml) in drinking water for 7 days. The solutions were replaced every day. The animals were allowed to drink the solution *ad libitum*. All the groups received the same standard nutrition and had the same living conditions for the 90-day period.

Parameters

Blood glucose (BG), glycosuria, ketonuria and body weight (BW) were determined before starting, daily in week 1, and once a week for the following 90 days.

Blood glucose was measured by nicking the tail and drawing a drop of blood that was assayed using a One Touch glucometer. Animals with BG level greater than 250 mg/dl in one determination or two BG levels greater than 200 mg/dl were considered diabetic, and were anesthetized by ether inhalation, exsanguinated and sacrificed.

Insulinemia was determined at the onset of the experiment and at 7, 30 and 90 days. Blood for this purpose was obtained by puncturing the retroauricular venous plexus and was drawn using heparinized capillaries and tubes containing EDTA. Insulinemia was assayed with a Rat Insulin ELISA DRG kit, (Instruments GmbH, Germany), using rat insulin as a standard. The ELISA sandwich technique utilizes two monoclonal antibodies preparations: anti-insulin antibodies conjugated with peroxidase and anti-insulin antibodies fixed to the microplate. The fixed conjugate was detected by means of 3,3',5,5' tetramethylbenzidine and read with the BioRad Reader at 450nm (reference filter 650nm).

Statistical Analysis

All results were expressed as mean and standard deviation of the mean. Comparisons between means were made using the Student's unpaired t test. A probability value of $p<0.05$ was considered to indicate a significant difference between means.

Results

The age of onset of diabetes

The age of onset of diabetes was significantly higher for groups V1 (84.8±16.8 days), V2 (102.8±33.5 days), V3 (105.5±16.1 days) compared to control group C (73.9±10.5 days), $p<0.05$ (V2, V3 vs. C), and depended on the metavanadate concentration (V3 vs. V1, $p=0.006$) (Fig. 1).

The incidence of diabetes

The incidence of diabetes was lower in the rats treated with metavanadate (V1: 50%, V2: 45.8%, V3: 45.8%) than in the rats from the control group (C: 54.16%), but this difference was not statistically significant ($p>0.05$).

Blood glucose level

The levels of blood glucose in groups V1, V2, V3 decreased slowly in week 1 (V1 from 95.6±2.5 mg/dl to 63±11.1 mg/dl, $p=0.007$, V2 from 100.3±2.5 mg/dl to 73±6.5 mg/dl, $p=0.0025$, V3 from 98±2.8 mg/dl to 73±5.6 mg/dl, $p=0.03$) and increased slightly in week 2 (V1: 98.5±7.7 mg/dl, V2: 97±1.4 mg/dl, V3: 101.5±4.9 mg/dl). In week 3, the blood glucose decreased again and remained at lower levels than in group C for the whole duration of the experiment (90 days).

In the rats diagnosed with diabetes, the blood glucose at the onset was higher in group C (383.2±48.2 mg/dl) than in groups treated with metavanadate (V1: 276.8±103.4 mg/dl, V2: 286.1±107.1 mg/dl), $p<0.05$ (V1, V2 vs. C) (Fig. 2).

Body weight

In the two weeks preceding the onset of diabetes, the rats from group C lost in body weight from 239.5±55.8 g to 208.5±34.6 g while the rats in groups V presented a slight increase in body weight from 309.4±23.5 g to 344±16.8 g (C vs. V1, V2, V3, $p<0.05$) (Fig. 3).

Plasma insulin

The first stage in evaluating serum insulin levels was to determine normal insulin levels in BB-DP rats (1.81 µg/l) and the insulin levels at which these animals develop diabetes (0.043-0.125 µg/l).

The levels of insulin at the start, at 7, 30 and 90 days in nondiabetic animals treated with metavanadate are shown in Fig. 4. At the end of the 7 days of administration, we observed a decrease in insulinemia proportional to the dose of vanadium (V1 1.78±0.18 µg/l to 0.43±0.08 µg/l, V2 from 1.75±0.16 µg/l to 0.38±0.08 µg/l and V3 from 1.75±0.18 µg/l to 0.27±0.07 µg/l). Afterwards, the insulin levels continued to rise until the end of the experiment and even exceed-

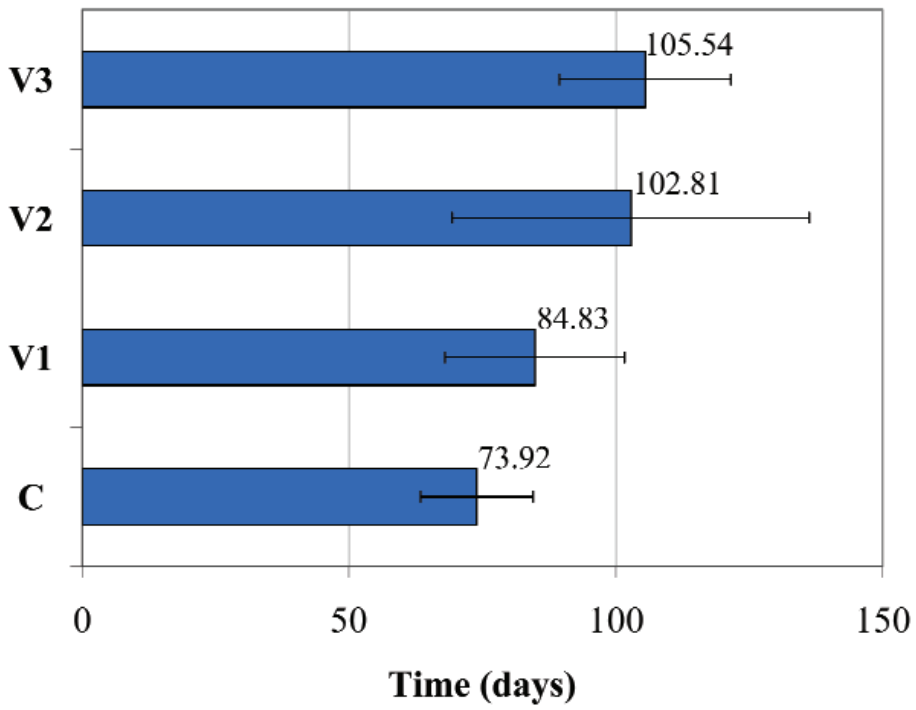

Fig. 1 The average age of onset of diabetes in the four groups (V2, V3 vs. C, $p<0.05$).

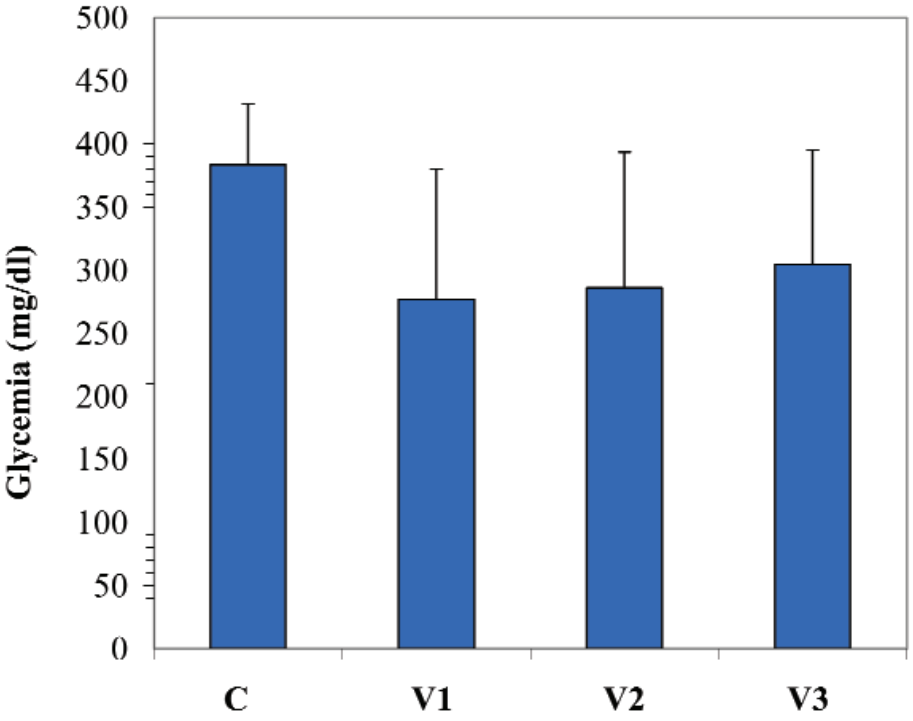

Fig. 2 The blood glucose levels at the onset of diabetes in the four groups (V1, V2 vs. C, $p<0.05$).

ed normal levels in the V2 group. On day 30, the following insulin levels were noted V1: 0.73±0.1 µg/l, V2: 1.17±0.17 µg/l and V3: 0.46±0.06 µg/l. On day 90, insulinemia was V1 1.15±0.16 µg/l, V2 2.89±0.3ig/l and V3 0.86±0.09 µg/l.

Discussion

In the present study, we aimed extending the observations related to the short-term treatment effects of metavanadate on prediabetic BB-DP rats (40-50 days of age), a strain of spontaneously diabetic

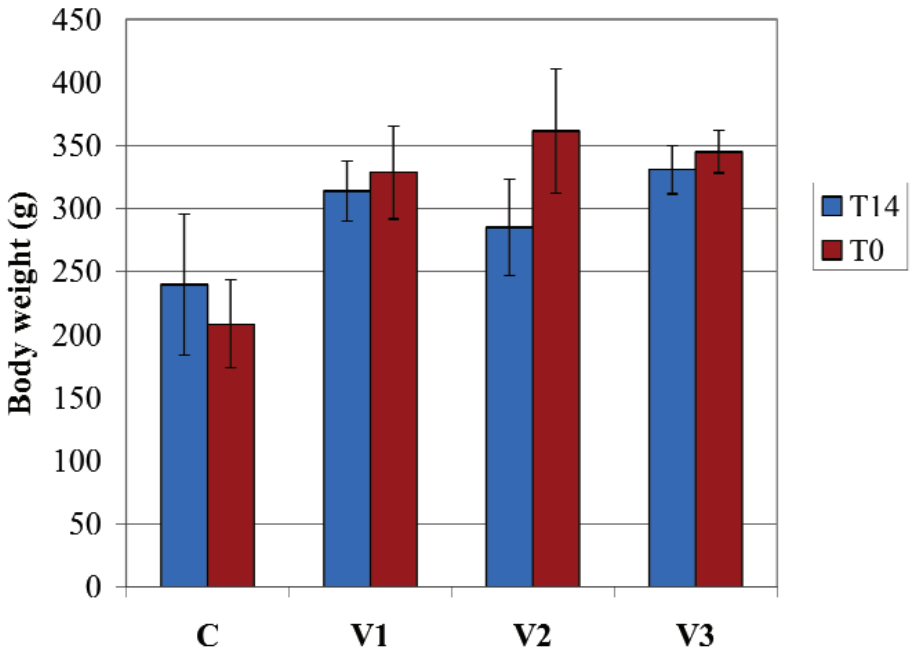

Fig. 3 The evolution of body weight two weeks before the diabetes onset (T14 - body weight two weeks before the diabetes onset; T0 - body weight at the diabetes onset).

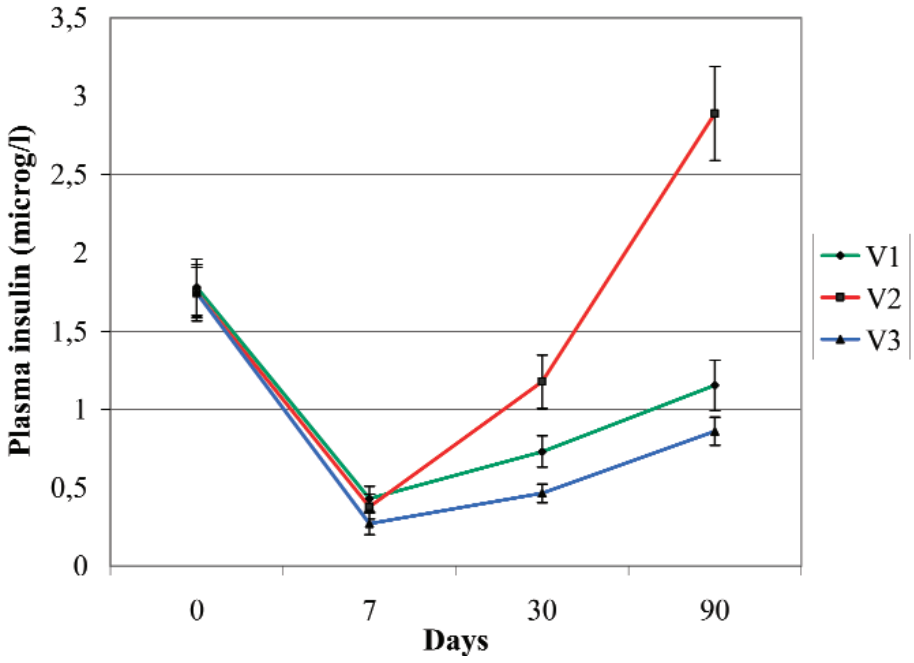

Fig. 4 The insulin levels in non-diabetic treated rats.

Wistar rats which develop a disease that closely resembles human type 1 diabetes. It is known that the BB-DP rats become diabetic at a rate of 60-80% with the onset at about 60 days of age, peaking at about 100 days of age. We report that sodium metavanadate delayed the development of diabetes, but did not prevent the onset of diabetes mellitus in BB-DP rats. Although the incidence of diabetes was lower in rats treated with metavanadate than in rats from control group, this difference was not statistically significant.

In groups treated with metavanadate, onset of diabetes was delayed on average by 30 ± 5.2 days compared to the control group. The age at the onset

of diabetes was correlated with metavanadate concentration, thus the greatest dose of metavanadate determined the greatest delay of diabetes.

The previous studies of Meyerovitch *et al.* showed that the oral administration of metavanadate to 8-week old prediabetic NOD mice for 18 weeks did not decrease the incidence of diabetes [15]. However, vanadium treatment was demonstrated to improve pancreatic insulin content in STZ-induced diabetic rats [16, 17]. It has been reported that the residual insulin reserves in diabetic rats following vanadium treatment were 5.6 fold [17] to 7.8 fold [16] higher than in untreated diabetic animals, suggesting that a reduction in insulin biosynthesis and secretion can render beta-cells less susceptible to cytotoxic events [7, 18, 19].

Since vanadium pretreatment did not prevent STZ-induced beta-cytotoxicity, the vanadium-induced amelioration of the diabetic state may be secondary to the preservation of a functional portion of pancreatic beta-cells that initially survive the STZ-induced beta-cytotoxicity [20]. Apparently, small changes in the islet insulin stores, in a model of reduced beta-cell mass, can have profound long-term consequences for glucose homeostasis [20].

In support of this theory, we already know that prophylactic insulin treatment prevents the onset of diabetes in genetically susceptible BB rats and NOD mice. Vlahos *et al.* showed that chronic prophylactic exogenous insulin treatment initiated in young diabetes-susceptible BB rats prevents type 1 diabetes, supporting the hypothesis that decreased beta-cell activity is responsible for protection against immune beta-cell destruction [14].

The data obtained from animal studies indicate that prophylactic insulin administration may offer protection against diabetes although this protection is not complete [1]. One explanation for the beneficial effect is "beta-cell rest" which results from a metabolic effect of insulin on the beta-cell. If the metabolic activity of the beta-cell is decreased, it may be less susceptible to specific immune damage [1]. Another explanation appears to be connected to the immunomodulatory effects of insulin as an important beta-cell antigen, since immunizing doses and B-chains were also effective [1]. Our results were in agreement with most of the literature. A group comprising 75 BB-DP rats received small doses of insulin (0.05, 0.1 and 0.2 IU of insulin/100 g body weight) while another group of 25 rats received normal saline for 60 days. The conclusion was that the administration of insulin could delay or even prevent the clinical onset of type 1 diabetes [2].

The hypoglycemic effect of sodium metavanadate caused a gradual drop in insulin in the first days of administration without reaching hypoglycemic levels or death from severe hypoglycemic accidents. The immediate effects of metavanadate on glycemic levels could be noted in the first days of administration. By comparison, the glycemic levels in the control group remained constant. In rats that became diabetic, the levels of glycemia were significantly higher in the untreated animals than in the group that received metavanadate (C vs. V1, V2). Bearing in mind that the glycemic levels recorded at the onset of disease suggest the degree of severity of the disease, we can therefore state that animals treated with sodium metavanadate developed a milder form of diabetes. Moreover, 2 weeks before the onset of diabetes, the rats in the control group lost approximately 13% of their weight while the rats that received vanadium experienced a mild weight gain of approximately 10% (Fig. 3). These results demonstrate both the anabolic effects of metavanadate as well as its effects on the amelioration of the severity of disease at onset.

It has been established that vanadium in non-diabetic animals does not significantly affect glycemia though it causes a decrease in plasma insulin concentration [21]. These findings suggest that the effects could be determined by the enhancement of tissue sensitivity to insulin [21, 22]. In the present study, insulinemia measured before and after treatment diminished in the treated groups in proportion to the dosage of the administered vanadium. After treatment, insulin levels increased gradually until the end of the experiment.

Thus, at the end of the 90 days of observation, the average insulin levels of the non-diabetic animals in group V2 (0.2 mg/ml) exceeded by 59.7% the normal levels, while in the other two groups, the average values of insulin were only 63.7% (V1 group) and 47.5% (V3 group) of the normal values. These effects may explain the long-term hypoglycemic action of vanadium salts, as well as their general anabolic effects as they could stimulate the synthesis of preproinsulin.

On the other hand, it is known that the hypoglycemic effects of vanadium are associated with

the altered expression of some genes involved in the glucose metabolism in the liver. Vanadium may mimic the action of insulin even at the genomic level by increasing the expression of the type L pyruvate kinase gene, inhibiting glucose-6-phosphatase, and reducing the level of phosphoenol pyruvate carboxykinase mRNA etc. [7, 10, 23–26].

Previous research showed that long-term treatment with vanadate increases the level of serum insulin in models of type 2 diabetes, such as the ob/ob rats [27] and the Zucker diabetic fatty rats [28]. Other studies found that vanadate treatment does not change plasma insulin levels, and even lowers them in non-diabetic rats [29]. Judging from the results we obtained, these earlier findings must be put into perspective, considering the duration of administration, the state of the animal and the moment in time when the data were recorded.

In our study, the levels of insulinemia remained relatively high for a long time. This can be justified through metavanadate insulin-sensitizing action that decreased the endogenous production of insulin. In turn, it delayed the rapid exhaustion of the pancreas, explaining as well the delay in the onset of the disease. The rise of insulin levels after treatment is at least partially due to stimulation of insulin release, acting directly on the beta-cell [30].

After the evaluation of several parameters (weight two weeks before the onset of diabetes, glycemia, age at onset of disease as well as insulin secretion) throughout the entire experiment, we can state that the best effects were observed in group V2 that received metavanadate in concentrations of 0.2 mg/dl. These observations suggest that the effects of metavanadate depend also on dosage. Meyerovitch *et al.* arrived at the same conclusion when they demonstrated that the optimum antidiabetic dose of metavanadate in streptozotocin rats was 0.2 mg/dl [31].

Taken together, our findings indicate that sodium metavanadate delays the development of diabetes mellitus in BB-DP rats, but it does not prevent its onset. It also determines the occurrence of a milder form of the disease by making the residual insulin more effective during the progressing pancreatic lesion. The effects depend on the sodium metavanadate concentration and 0.2 mg/ml is preferrable.

Research spanning over 20 years on the insulin-like action of vanadium salts have led to the conclusion that they could be used as a substitute for insulin in the future treatment of diabetes. Nevertheless, there is need for a complete evaluation of secondary effects of inorganic and organic vanadium compounds, prior to its introduction in human therapeutics. The most important could be, at present, the elucidation of the mechanisms of the action of diverse vanadium salts, including interferences in the signaling pathways of insulin and the study of the influence of vanadium at genetic level.

References

1. **Cheta D.M.**, Preventing Diabetes, Theory, Practice and New Approaches, Wiley, Chichester, 1999
2. **Cheta D., Orasanu G.**, Prevention of type 1 diabetes in animal models. In: Cheta D. ed., New Insights into Experimental Diabetes, The Publishing House of the Romanian Academy, Bucharest, 2002, pp. 362-403
3. **McNeill J.H., Yuen V.G., Hoveyda H.R., Orvig C.**, Bis(maltolato)oxovanadium (IV) is a potent insulin mimic, *J. Med. Chem.*, **35**:1489-1491, 1992
4. **Ramanadham S., Brownsey R.W., Cros G.H., Mongold J.J., McNeill J.H.**, Sustained prevention of myocardial and metabolic abnormalities in diabetic rats following withdrawal from oral vanadyl treatment, *Metabolism*, **38**:1022-1028, 1989
5. **Morinville A., Maysinger D.**, Shaver A., From vanadis to atropos: vanadium compounds as pharmacological tools in cell death signaling, *Trends Pharmacol. Sci.*, **19**:452-460, 1998
6. **Dai S., Thompson K.H., Vera E., McNeill J.H.**, Toxicity studies on one-year treatment of non-diabetic and streptozotocin diabetic rats with vanadyl sulphate, *Pharmacol. & Toxicol.*, **75**:265-273, 1994
7. **Brichard S.M., Henquin J.C.**, The role of vanadium in the management of diabetes, *Trends Pharmacol. Sci.*, **16**:265-270, 1995
8. **Li S.H., McNeill J.H.,** In vivo effects of vanadium on GLUT4 translocation in cardiac tissue of STZ-diabetic rats, *Mol. Cell. Biochem*., **217**:121-129, 2001
9. **Wang J., Yuen V.G., McNeill J.H.,** Effect of vanadium on insulin and leptin in Zucker diabetic fatty rats, *Mol. Cell. Biochem*., **218**:93-96, 2001
10. **Shafrir E., Spielman S., Nachliel I., Khamaisi M., Bar-On H., Ziv E.,** Treatment of diabetes with vanadium salts: general overview and amelioration of nutritionally induced diabetes in the *Psammomys obesus gerbil*, *J. Clin. Endocrinol. Metab*., **86**:1410-1417, 2001
11. **Posner B.I.**, Peroxovanadium compounds: potent PTP inhibitors with insulin-like effects [Abst.], The EASD Satellite Symposium, Sitges, Barcelona, **9**:2000
12. **Cam M.C., Rodrigues B., McNeill J.H.**, Distinct glucose lowering and beta cell protective effects of vanadium and food restriction in streptozotocin-diabetes, *Eur. J. Endocrinol.*, **141**:546-554, 1999

13. **Sekar N., Li J., Shechter Y.**, Vanadium salts as insulin substitutes: mechanism of action, a scientific and therapeutic tool in diabetes mellitus research, *Crit. Rev. Biochem. Mol. Biol.*, **31**:339-359, 1996
14. **Vlahos W.D., Seemayer T.A., Yale J.F.**, Diabetes prevention in BB rats by inhibition of endogenous insulin secretion, *Metabolism*, **40**:825-829, 1991
15. **Meyerovitch J., Waner T., Sack J., Kopolovic J., Shemer J.**, Attempt to prevent the development of diabetes in non-obese diabetic mice by oral vanadate administration. *Isr. Med. Assoc. J.*, **2**:211-214, 2000
16. **Blondel O., Bailbe D., Portha B.**, *In vivo* insulin resistance in streptozotocin diabetic rats - evidence for reversal following oral vanadate treatment, *Diabetologia*, **32**:185-190, 1989
17. **Brichard S.M., Okitolonda W., Henquin J.C.**, Long term improvement of glucose homeostasis by vanadate treatment in diabetic rats, *Endocrinology*, **123**:2048-2053, 1988
18. **Yilmaz M.T.**, The remission concept in type 1 diabetes and its significance in immune intervention, *Diabetes. Metab. Rev.*, **9**:337-348, 1993
19. **Sprietsma J.E., Schuitmaker G.E.**, Diabetes can be prevented by reducing insulin production, *Med. Hypotheses*, **42**:15-23, 1994
20. **Cam M.C., Li W.M., McNeill J.H.**, Partial preservation of pancreatic beta-cells by vanadium: evidence for long-term amelioration of diabetes, *Metabolism*, **46**:769-778, 1997
21. **Dai S., Thompson K.H., McNeill J.H.**, One year treatment of streptozotocin-induced diabetic rats with vanadyl sulphate, *Pharmacol. & Toxicol.*, **75**:101-109, 1994
22. **Cam M.C., Brownsey R.W., McNeill J.H.,** Mechanisms of vanadium action: insulin-mimetic or insulin-enhancing agent?, *Can. J. Physiol. Pharmacol.,* **78**:829-847, 2000
23. **Brichard S.M., Desbuquois B., Girard J.**, Vanadate treatment of diabetic rats reverses the impaired expression of genes involved in hepatic glucose metabolism: effects on glycolytic and gluconeogenic enzymes and on glucose transporter GLUT2, *Mol. Cell. Endocrinol.*, **91**:91-97, 1993
24. **Mosseri R., Waner T., Shefi M., Shafrir E., Meyerovitch J.**, Gluconeogenesis in non-obese diabetic (NOD) mice: in vivo effects of vanadate treatment on hepatic glucose-6-phoshatase and phosphoenolpyruvate carboxikinase, *Metabolism*, **49**:321-325, 2000
25. **Ramachandran B., Kandaswamy M., Narayanan V., Subramanian S.,** Insulin mimetic effects of macrocyclic binuclear oxovanadium complexes on streptozotocin-induced experimental diabetes in rats, *Diabetes. Obes. Metab.,* **5**:455-461, 2003
26. **Cusi K., Cukier S. DeFronzo R.A., Torres M., Puchulu F.M., Redondo J.C.,** Vanadyl sulfate improves hepatic and muscle insulin sensitivity in type 2 diabetes, *J. Clin. Endocrinol. Metab.,* **86**:1410-1417, 2001
27. **Meyerovitch J., Rothenberg P., Shechter Y., Bonner-Weir S., Kahn KR.**, Vanadate normalizes hyperglycemia in two mouse models of non-insulin dependent Diabetes Mellitus, *J. Clin. Invest.*, **87**:1286-1294, 1991
28. **Wang J., Yuen V.G., McNeill J.H.,** Effect of vanadium on insulin and leptin in Zucker diabetic fatty rats, *Mol. Cell. Biochem.,* **218**:93-96, 2001
29. **Malabu V.H., Dryden S., McCarthy H.D., Killpatrick A., Williams G**., Effects of chronic vanadate administration in the STZ-induced diabetic rat. The antihyperglycemic action of vanadate is attributable entirely to its suppression of feeding, *Diabetes*, **43**:1267-1270, 1994
30. **Conconi M.T., DeCarlo E., Vigolo S., Grandi C., Bandoli G., Sicolo N., Tamagno G., Parnigotto P.P., Nussdorfer G.G.,** Effects of some vanadyl coordination compounds on the in vitro insulin release from rat pancreatic islets, *Horm. Metab. Res*., **35**:402-406, 2003
31. **Meyerovitch J., Farfel Z., Sack J., Shechter Y.**, Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats: characterization and mode of action, *J. Biol. Chem.*, **262**:6658-6662, 1987