Codelivery of a Tea Extract Prevents Morbidity and Mortality Associated With Oral Vanadate Therapy in Streptozotocin-Induced Diabetic Rats

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Oral administration of vanadate has a strong hypoglycemic effect but results in toxic side effects like life-threatening diarrhea. Tea is known to have potent antidiarrhea effects. We investigated the potential of suspending the vanadate in a tea decoction to reduce the diarrhetic action of vanadate. A concentrated extract of Lichee black tea was, therefore, added to sodium orthovanadate. Streptozotocin (STZ)-induced diabetic rats were orally gavaged with vanadate suspended in water or in the tea decoction, or with the tea extract alone. Blood glucose levels were assessed daily over 11 weeks with levels greater than 10 mmol/L warranting therapeutic intervention. Both the vanadate/water and vanadate/tea solutions acutely reduced blood glucose. The tea extract alone had no effect. The majority of vanadate/water-treated rats developed diarrhea and mortality rates approached 40%. Vanadate/tea-treated diabetic rats experienced no diarrhea or mortality and liver and kidney analyses (plasma ALT and creatinine, blood urea nitrogen [BUN], and urine-specific gravity) were normal. Animals treated with vanadate/tea retained blood glucose levels less than 10 mmol/L for an average of 24 consecutive days without subsequent treatments. Cataract formation was completely prevented. The mechanism of action of vanadate may have involved β-cell stimulation because vanadate/tea-treated diabetic rats exhibited normal plasma insulin levels. In summary, because of its long-lasting effects, oral administration, and lack of side effects, vanadate/tea represents a potentially important alternative therapy for an insuluin-defecient diabetic state.

TYPE I DIABETES is a disease characterized by a deficiency of endogenous insulin. In addition, circulating glucose levels are elevated and many metabolic processes are abnormal.[1-4] The disease generates cardiovascular, renal, ocular, and nervous system complications.[5-11] Rates of cardiovascular disease in diabetic patients are 3-fold higher than that encountered in the nondiabetic population, and cardiovascular disease is the leading cause of mortality.[12] Diabetes-induced peripheral neuropathy alone results in a 3- to 4-fold increase in lower leg amputations.[13] Similarly, early onset of cataracts and kidney failure significantly impact both morbidity and mortality rates associated with diabetes.[8]

The discovery of insulin by Banting and Best in 1921 represented a significant advance in the treatment of diabetes.[14,15] This enabled physicians to control the glucose intolerance in type I diabetic patients. However, the medical, financial, and social burdens of diabetes continue to rise.[16] Therefore, the search continues for more effective treatments capable of both lowering blood glucose and controlling diabetic complications.

In this search for a new hypoglycemic agent, vanadium compounds have received much attention.[17-21]. Vanadium is an ultratrace element found both in the body and the environment. A clear understanding of its biologic function has not been achieved to date. In its oxidized state (vanadate), it has been reported to reduce blood glucose levels and restore cardiac function in insulin-deficient diabetic rat models.[22,23] Vanadate has been suggested to work through both insulin receptor–independent and –dependent mechanisms to control blood sugar levels.[17-21] For example, vanadate has been suggested to stimulate insulin release from the pancreas.[24] Alternatively, vanadate may have an intracellular site of action in peripheral tissues. An intracellular phosphorylation process is critical for the movement of the glucose transporter (GLUT 4) to the cell surface to induce glucose transport. Vanadate is a potent phosphatase inhibitor.[25-27] Thus, phosphorylation and GLUT 4 movement would be stimulated by phosphatase inhibition. However, the promising effects found with respect to the reduction of blood glucose levels have been offset by major side effects affecting the gastrointestinal tract. These have included reduced food intake in treated animals and severe diarrhea resulting in mortality.[28-30] Effective hypoglycemic doses of vanadium have produced similar gastrointestinal effects in small human trials and thus limited the clinical use of vanadium to date.[31,32]

Clearly, if the gastrointestinal complications associated with vanadium could be controlled, this compound would represent a significant therapeutic agent in the regulation of diabetic symptoms. Previously, it has been shown that a variety of different nutraceuticals or functional foods have gastrointestinal protective effects.[33] These include but are not limited to tea, rhatany root, tormentil, bilberry fruit, oak bark, wild strawberry leaf, silverweed, agrimony, chestnut leaf, red sage, and walnut leaf. With the widespread acceptance of alternative therapeutics and the proven hypoglycemic actions of vanadium, we sought a combination that could yield both antidiabetic and antidiarrhetic properties. The purpose of our study, therefore, was to test a novel compound that contains sodium orthovanadate in a black tea extract for its antidiabetic efficacy in an animal model of chronic insulin-dependent diabetes mellitus.

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Submitted October 29, 2003; accepted March 7, 2004.

Supported in part by the St. Boniface Hospital Research Centre and CIHR. G.N.P. is a CIHR Senior Scientist.

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0026-0495/04/5309-0005$30.00/0

doi:10.1016/j.metabol.2004.03.017

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 175 to 200 g were obtained from Central Animal Care Services at the University of Manitoba (Winnepeg, Canada). Animals were housed on woodchip bedding in polycarbonate cages, and offered free access to both food (lab diet 5P00, Prolab, Brentwood, MO) and water. A 12:12 light:dark cycle was employed using 6 AM to 6 PM as the light cycle. The animals were maintained at 20°C with 50% humidity throughout the study. The experimental period was 11 weeks. Animals were killed with a single intraperitoneal injection of a 9 mg/mL ketamine:0.9 mg/mL xylazine cocktail. Blood was collected by exsanguination and centrifuged briefly to obtain plasma. Plasma was stored at −20°C for subsequent analysis.

Insulin-Dependent Diabetic Model

Rats were lightly anesthetized with 2% isoflurane and 2% O_2 gas and tail vein injections of streptozotocin (STZ) were used for diabetic induction. STZ at a dosage of 55 mg/kg body weight was diluted in a citrate buffer vehicle (pH 4.5) and injected in a single dose as described previously.[34,35] Control animals received an injection of buffered vehicle alone. Animals were allowed to adjust to their diabetic state for 4 days prior to any treatment.

Blood Glucose Analysis

Four days post-STZ administration, blood glucose levels were assessed in all animals using a Bayer Glucometer Elite testing system (Etobicoke, Ontario, Canada). A distal tail snip generated the 5 μL quantity of blood necessary for analysis. Daily glucose levels were determined at 9 AM by removing the scab formed on the tail.

Treatment of Diabetes

A decoction of sodium orthovanadate (Sigma, St Louis, MO) was made by suspending 20 mg vanadate/mL tea extract. The tea extract was prepared by bringing 1.0 L of double-distilled water to a boil and then adding 60 g of Chinese Lichee Black Tea leaves (Golden Sail brand, China Tuhsu Guangdong Tea Import and Export Corp, China). The heat was reduced, and the extract was protected from light and allowed to simmer for 10 to 15 minutes with frequent stirring. The extract was allowed to cool for 2 hours before filtering it through 2 layers of cheesecloth. The filtered solution was refiltered through 4 layers of cheesecloth and can be stored at 4°C for up to 7 days. The vanadate/tea was made with 20 mg of sodium orthovanadate added to each 1-mL aliquot of Lichee Black Tea. The sodium orthovanadate was suspended into the tea with stirring. The solution was then stored in the dark at room temperature until ready for use approximately 5 hours later. Any unused vanadate/tea was discarded. Fresh solution was made daily. The choice of Lichee Black tea as the solvent of choice was made on the basis of preliminary trials with a number of different teas (data not shown; see Discussion). In order to properly compare the results of vanadate/tea to the conventional vanadate preparations used in the past, a water/vanadate preparation was also used to treat the diabetic animals. Sodium orthovanadate was suspended in double-distilled water at a concentration of 20 mg/mL and stored in the dark at room temperature for 5 hours prior to animal treatment.

Any animal with a blood glucose level greater than 10 mmol/L was orally gavaged at 4 PM daily with the appropriate treatment solution. Rats with daily blood glucose levels less than 10 mmol/L were considered to have glycemic levels that were only slightly above control levels (~7 mmol/L), manageable, and not treated that day. Animal treatment groups included vanadate/tea-treated diabetics (tea/van-D) (n = 15), water/vanadate-treated diabetics (water/van-D) (n = 13), diabetic rats (D) treated with the black tea extract alone (without vanadate) (n = 5), and nondiabetic animals (ND) (n = 5). Each animal received 2 mL of treatment solution, corresponding to a total vanadium dose of 40 mg.

Plasma/Urine Analysis

Biochemical diagnostic kits (Sigma) were used for the assessment of ALT, triglycerides (TG), and cholesterol levels within plasma. A Vet test 8008 spectrophotometer (IDEXX Laboratories) was used for blood urea nitrogen (BUN) and creatinine analysis. Urine specific gravity was assessed with a hand refractometer (Atago, Tokyo, Japan) while plasma insulin levels were measured with an enzyme-linked immunosorbent assay (ELISA).

Vanadium Level Determination: Sample Preparation

Plasma samples were diluted 1:1 with 0.25 mol/L sodium citrate solution containing 1% Triton X-100. These solutions were directly assayed for vanadium. Weighed aliquots of frozen tissue samples were digested in 3 stages: the first using 4 mL concentrated HNO_3, the second using a combination of 2 mL HNO_3 and 30% H_2O_2, and finally using 2 mL HNO_3. All digestions were performed at 130°C until complete drying was achieved. After the third drying, 1% HNO_3 was added to the digests and heated at 80°C for 1 hour. Once cool, the samples were recorded for volume and analyzed. Dilutions were made as required. Standard solutions of vanadium at varying concentrations were prepared from a Certified Reference Standard of vanadium (1 mg/mL, SCP Science Baie D'Urfe, Quebec, Canada). Calibration curves were generated before and after sample sets. Standard solutions from 0 to 100 μg/L for plasma analysis were made up in a 0.125 mol/L sodium citrate (Fisher Scientific, Nepean, Ontario, Canada) solution containing 0.5% Triton X-100 (BDH, Toronto, Ontario, Canada). Standard solutions from 0 to 100 μg/L for tissue analysis were made up in 1% HNO_3. Vanadium concentrations were measured using a Polarized Zeeman Graphite Furnace Atomic Absorption Spectrophotometer (Wellesley, MA) equipped with an autosampler. The detector wavelength was set to 318.4 nm using a slit width of 0.40 nm. A vanadium lamp current of 10 mA was used. A deuterium lamp was used for background correction. Sample volumes of 20 μL were used followed by insertion into a graphite tube. The following temperature profile was used for vanadium analysis: 2 drying steps beginning at 80 to 120°C ramped over 30 seconds with a hold time of 20 seconds, and from 120 to 450°C ramped over 25 seconds and held for 20 seconds. This was followed by an ashing step starting at 900 to 1,400°C ramped for 25 seconds and held for 20 seconds, and finally atomization at 2,850°C for 10 seconds. A 10-second cleaning step at 3,000°C as well as a 5-second cool down were programmed for each run. The purge gas used in these analyses was argon used at a flow rate of 200 mL/min, except during atomization at which time it was set to 40 mL/min. Vanadium concentrations were calculated from external standards based on relative correlations in peak absorbance. Instrument performance and result validity was obtained through sample spikes, standard re-runs, and for the tissue samples, digested blanks and digested bovine liver 1577b (Standard Reference Material). The low-end detection limit of the instrument was 2.0 μg/L. All analyses were carried out in duplicate.

Statistical Analysis

Statistical treatment of data was performed using an analysis of variance (ANOVA) test followed by a Duncan's post hoc test. Results are reported as the mean ± SE. Statistical significance was determined at $P < .05$.

RESULTS

The incidences of diarrhea and mortality for the water/van-D and tea/van-D animals are presented in Fig 1. Approximately

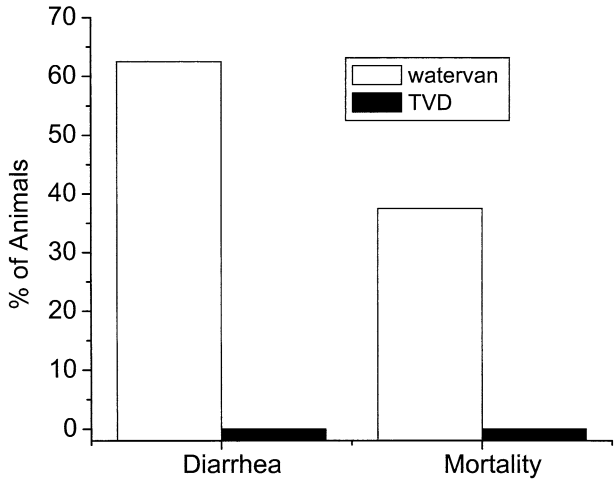

Fig 1. Incidence of diarrhea and death in diabetic rats administered sodium orthovanadate with water (watervan) or with tea (TVD). Values represent the % of total number of animals experiencing these parameters; n = 8 and 15 in the watervan and vanadate/tea groups, respectively.

63% of the water/van-D animals developed severe diarrhea and 38% of the water/van-D animals died as a direct result of these complications. By comparison, tea/van-D animals were completely free of both diarrhea and mortality.

Blood glucose levels were monitored throughout the study. The results are shown in Fig 2. Pretreatment glucose levels in all of the diabetic animal groups were approximately 22 mmol/L. Untreated D animals showed a gradual increase in blood glucose concentration rising from 22.5 mmol/L to 25 mmol/L over the 11-week experimental period. By comparison, ND animals had blood glucose levels of 5 to 7 mmol/L throughout the study. After the induction of diabetes with the STZ injection, the first treatment was administered on day 4 and this reduced blood glucose levels in the tea/van-D animals to control levels (~7 mmol/L) within 24 hours. This was found in both water/van-D and tea/van-D animals and was statistically different compared to the D rats. The total dosage of vanadate delivered to the diabetic animals over the course of the study was similar whether administered with tea or with water. As long as blood glucose levels remained below the arbitrary value of 10 mmol/L, animals did not receive any additional treatment to control the blood glucose levels. Glucose stabilization over the 11 week study was maintained at a level of approximately 8 mmol/L in the 2 treated groups. This closely resembled the ND blood glucose values. The results obtained from the small number of water/van-D surviving rats were judged to be biased without a complete population to analyze. In addition, it was not considered ethical by the local animal care committee at our university to increase the initial pretreatment sample size of this group in another set of experiments in order to increase the population of surviving rats (in anticipation of the large number of animal deaths). Therefore, because of the toxic effects of water/vanadate, further analysis of surviving D rats in this treatment group was limited to the analysis of the hypoglycemic effects and tissue vanadium level upon termination of the study.

One of the most striking findings in this study was the stable, relatively low blood glucose levels established in the tea/van-D rats. These animals averaged 24 ± 6 consecutive days with blood glucose levels less than 10 mmol/L after each treatment. Therefore, for approximately one third of the 11-week study, blood glucose levels remained below 10 mmol/L without further vanadate/tea treatment. Blood glucose values remained below 10 mmol/L in individual rats from 4 to 71 consecutive days without receiving further vanadate/tea treatment. Fifty percent of the tea/van-treated diabetic animals exhibited glucose levels less than 10 mmol/L for more than 15 consecutive days without receiving further vanadate/tea treatment.

Another measure of the long-lasting hypoglycemic efficacy of vanadate/tea is the frequency of treatments each rat received over the course of the study. On average, the tea/van-D animals were treated only 17% of the days in this study. This amounts to 1 treatment every 6 days in order to maintain the daily blood glucose level of approximately 8 mmol/L in the tea/van-D animals.

The potential for vanadate/tea to have toxic side effects was investigated in greater detail. Vanadate delivered in water has been shown to inhibit food and water consumption. It was possible, therefore, that vanadate/tea may affect these parameters as well. Both food and water intake were measured in the ND, D, and tea/van-D animals during the study (Fig 3). ND animals consumed approximately 67 mL of water and 25 g of rat chow daily. As expected, D rats consumed significantly more water and food (175 mL of water and 45 g of rat chow daily). Tea/van treatment normalized water and food consumption. Water and food intake in tea/van-D animals was 65 mL and 25 g, respectively.

As shown in Fig 4, D rats had significantly lower body weights during the course of this study than their ND counter-

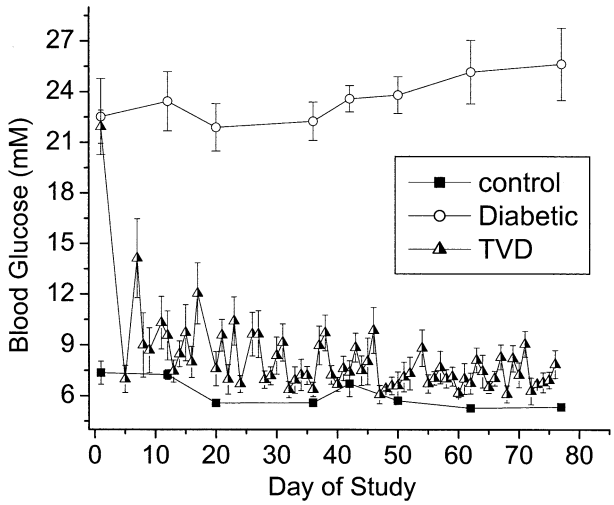

Fig 2. Blood glucose levels in nondiabetic (ND) control rats, diabetic (D) rats, tea/vanadate-treated diabetic (TVD) rats, and water/vanadate-treated (watervan) diabetic rats. Values represent mean ± SE of n = 5, 5, 15, 8, respectively, in each group. *$P < .0001$ *v* ND, TVD, and watervan.

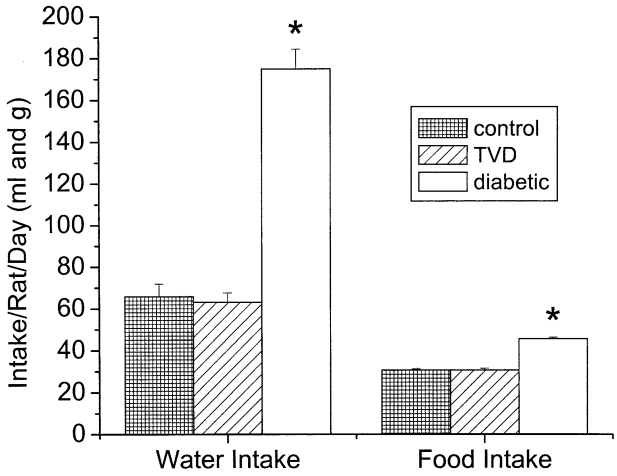

Fig 3. Water and food consumption in nondiabetic (ND) rats, diabetic (D) rats, and vanadate/tea-treated (TVD) diabetic rats. Intake of water (mL) and rat chow (g) was measured over a 24-hour period. Data are mean ± SE of n = 5, 5, 15, respectively. *$P < .05$ *v* ND and TVD.

parts. Vanadate/tea treatment did not normalize body weight. However, the rate of weight gain was greater in tea/van-D than in D rats. We chose to examine weight gain from days 20 to 30 because this represented a relatively linear increase ($R^2 = 0.97$) in weight over time. The control animals gained 4.50 g/d, the tea/van-D animals gained 3.61 g/d, and the untreated diabetic animals gained 1.43 g/d over this 10-day period of time.

Several additional parameters of toxicity and organ function were assessed (Table 1). Liver toxicity was measured with plasma ALT enzyme assays. Plasma ALT levels in ND rats were 50 U/L, whereas D animals had elevated ALT activity (64 U/L). ALT activity in tea/van-D rats was normalized (51 U/L). Kidney function was evaluated using the plasma creatinine assay, BUN analysis, and measurement of urine specific gravity. Creatinine levels were significantly lower in the D animals in comparison to the ND rats. Tea/van-D rats had creatinine levels that were significantly improved versus the D animals and not significantly different than ND values. BUN levels were not significantly different among the 3 groups. The specific gravity of urine was significantly higher in D rats in comparison to ND rats. Tea/vanadate treatment returned this value to control levels.

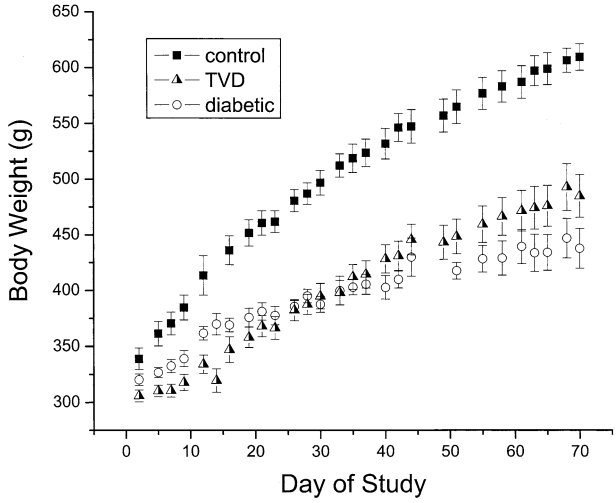

Fig 4. Body weights of nondiabetic (ND) rats, diabetic (D) rats, and vanadate/tea-treated (TVD) diabetic rats during the 11-week experimental period. Values represent mean ± SE of n = 5, 5, 15, respectively. Y = weight change as a function of time.

Table 1. Biochemical Analyses and the Incidence of Cataracts in Nondiabetic, Diabetic, and Tea/Vanadate-Treated Diabetic Rats

Analyses	ND	*D*	Tea/Van = D
TG (mg/dL)	150 ± 15	250 ± 68	198 ± 22
Cholestorol (mg/mL)	61 ± 5	83 ± 18	58 ± 5
Creatinine (mg/dL)	0.31 ± 0.02	0.23 ± 0.03*	0.31 ± 0.02
BUN (mg/dL)	17 ± 1	18 ± 1	17 ± 2
ALT (U/L)	50 ± 2	64 ± 4*	51 ± 5
Urine specific gravity	1.01 ± 0.003	1.03 ± 0.005*	1.02 ± 0.005
Insulin nol/L	0.69 ± 0.17	0.29 ± 0.01*	0.54 ± 0.08
Cataracts (%)	0	40*	0

NOTE. Data are means ± SE of n = 5, 5, 15 in ND, D, and Tea/Van-D rats respectively.

*$P < .05$ *v* ND animals.

Plasma cholesterol and TG were also measured in the rats (Table 1). Lipid levels tended to be higher in D rats when compared to ND values. However, this did not achieve statistical significance. Tea/vanadate treatment of D rats brought these indices closer to control values. We also monitored the status of plasma insulin as a function of diabetes and tea/vanadate treatment (Table 1). As expected,[5] plasma insulin levels were very low in D rats as a result of the STZ administration (~42% of control). Tea/van-D rats had plasma insulin levels similar to the ND animals and significantly improved compared to the D rats.

During the course of the study, cataract formation was observed in the diabetic rats. This is not unusual.[35] Although this was not analyzed via optical methods, the cataracts were easily visible as large areas of opaque cloudiness in the eye (Fig 5). Forty percent of the untreated diabetic rats had developed this lesion by the end of the study (Table 1). None of the ND controls or the tea/van-treated diabetic rats exhibited any visual evidence of cataract formation.

Finally, vanadium levels were measured in the ND, D, water/van-D, and tea/van-D animals following the study (Fig 6). All tissues measured (plasma, lungs, liver, bone, kidney, pancreas, and heart) in the ND and D animals yielded concentrations of vanadium lower than the detection limit of the spectrophotometer ([V] < 5.00 μg/L). As expected, levels in both the water/van-D and tea/van-D animals were elevated. In all organs and plasma, no significant differences were found between the 2 groups. The highest concentrations of vanadium were found in bone >kidney >liver >lung >pancreas >heart (Fig 6).

DISCUSSION

The lack of tightly regulated glycemic control in diabetes results in significant morbidity and mortality.[16] Presently, the conventional pharmacologic treatment for type I diabetes is

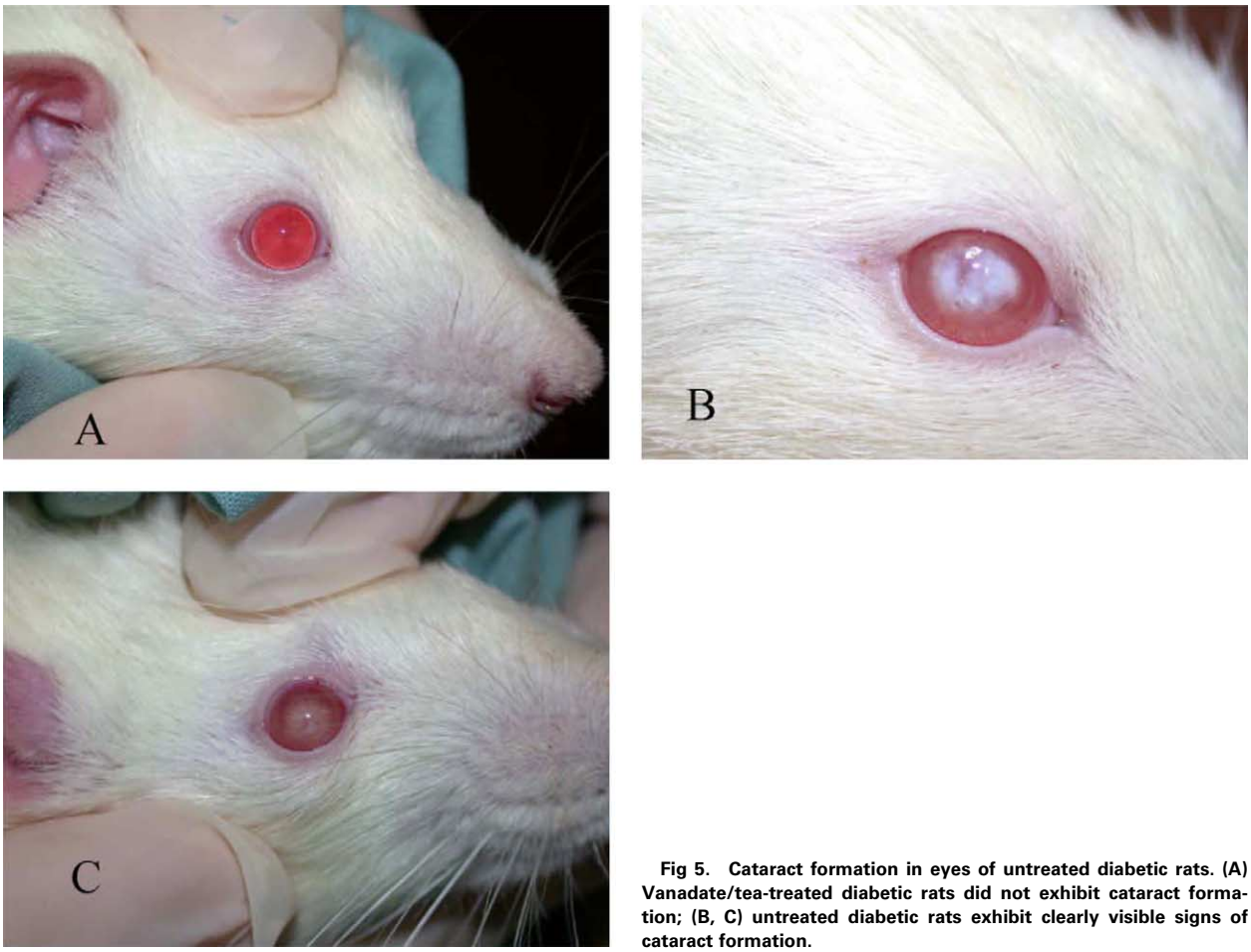

Fig 5. Cataract formation in eyes of untreated diabetic rats. (A) Vanadate/tea-treated diabetic rats did not exhibit cataract formation; (B, C) untreated diabetic rats exhibit clearly visible signs of cataract formation.

insulin therapy. Although intramuscular injections of insulin are effective in acutely reducing blood glucose levels, an oral delivery method for controlling blood glucose levels would minimize patient discomfort, eliminate needle costs and disposal concerns, and reduce chances of infection. Additionally, the fluctuating blood sugar levels that can be associated with insulin therapy necessitate monitoring and treatment more than once per day.

The ability of vanadate/tea to reduce blood glucose levels both acutely and chronically is apparent from the data shown in Fig 2. With a single dose of vanadate/tea, glucose levels drop in diabetic animals to nondiabetic control levels. More importantly, this glycemic control is sustainable over a period of several days and in some cases several weeks without subsequent treatment. Therefore, in addition to its mode of delivery, the capacity of vanadate/tea to regulate blood glucose levels over extended periods of time represents another exciting advantage of this therapy over the conventional insulin injection method.

Another risk associated with injected insulin is hypoglycemic shock. Continuous monitoring of drug dosage and blood glucose levels is mandatory. In this study, the 40-mg vanadate/tea dose given to the D animals was effective in controlling glucose levels long-term and was not associated with a single hypoglycemic event (reducing blood glucose <3 mmol/L). Over the 11 weeks of study, the dose of 20 mg vanadate/mL tea (40 mg in total) did not have to be increased despite large gains in animal body mass. This may certainly be important when the goal of eliminating side effects is as essential as it is with vanadium.

With blood glucose levels tested daily and tea/vanadate-treated animals requiring an average of only 1 dose every 6 days, one might speculate that secondary complications common with diabetics may be minimized with vanadate/tea treatment. Indeed, by the end of the study 40% of D rats had developed severe cataracts, a common complication of uncontrolled hyperglycemia[35] (Table 1). However, none of the tea/van-D rats exhibited cataracts. This further substantiates the strong hypoglycemic effect of the vanadate/tea compound.

Vanadium usage as a therapeutic clinical agent has been limited to date because of its deleterious side effects.[30-32] The elimination of both diarrhea and mortality in the tea/van-D animals suggest that the antidiarrhetic properties of the black tea extract work synergistically with the hypoglycemic action of sodium orthovanadate (Fig 1). This is the first known vanadium compound to express these characteristics. The mechanism whereby the Lichee Black tea exerts these protective effects is unclear. Not all tea decoctions exerted a similar

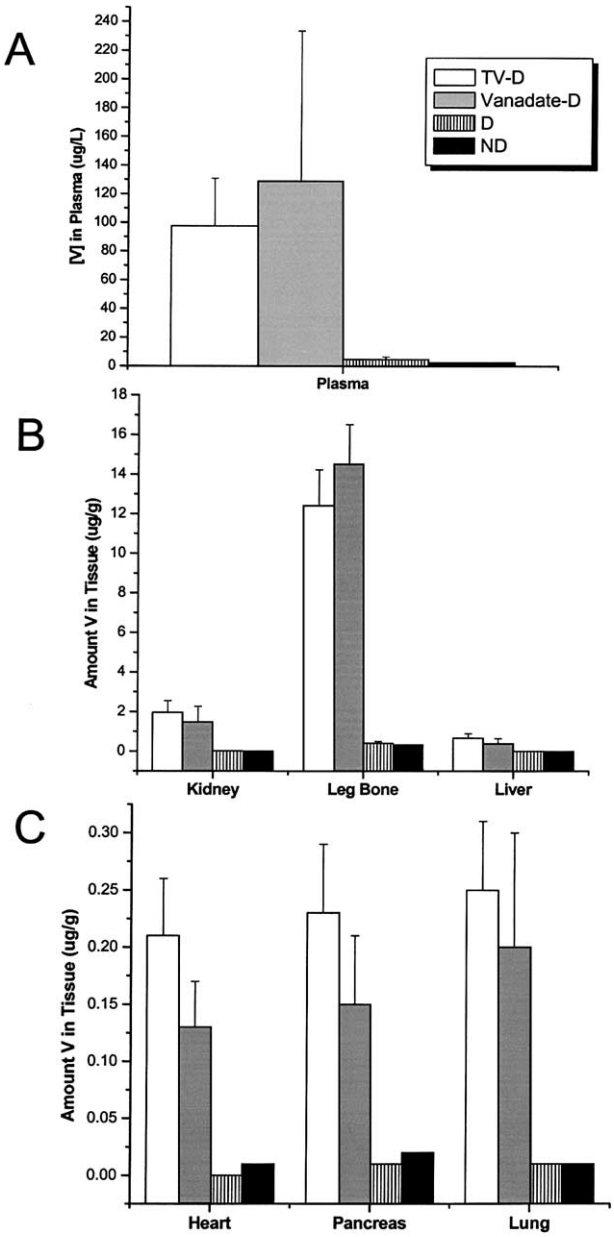

Fig 6. Vanadium levels in (A) plasma; (B) kidney, bone, and liver; and (C) heart, pancreas, and lungs from vanadate/tea-treated diabetic (TV-D), vanadate/water-treated diabetic (Vanadate-D), diabetic (D), and nondiabetic (ND) Sprague-Dawley rats. Data represent mean ± SE of n = 8, 5, 5, 7, respectively. There were no significant differences between values obtained from vanadate/tea-treated diabetic rats and vanadate/water-treated diabetic rats.

protective effect as Lichee Black tea. Jasmine and Japanese Green tea, but not Chinese Green tea or Mate tea, have been shown in preliminary studies to provide similar protective effects against vanadate-induced diarrhea in diabetic rats as the Lichee Black tea (data not shown). This would suggest that a specific component or a combination of ingredients within tea is important. However, our attempts to fractionate the tea have been unsuccessful and our data suggest that a synergistic effect of more than one component within the tea is providing the protective action. Tea is known to be unusually complex, containing thousands of individual components.[33] It is possible that the tea alters the chemical characteristics of the vanadate in solution or binds to it to negate its toxic effects in the gastrointestinal tract. Ultimately, these ingredients may alter the bioavailability of the vanadate. However, vanadate concentrations in the blood and tissue were similar when the vanadate was delivered with the tea extract as opposed to the conventional water vehicle. In any case, vanadate/tea not only prevented the toxicity normally associated with the gut, but all other assays for nonspecific tissue toxicity were negative (Table 1). Significant improvements in plasma creatinine, urine specific gravity, and normal ALT levels in tea/van-D animals suggest metabolic normalization, in addition to extended glycemic control. Although body weight was not normalized by the end of the study, water and food intake were normal and it would be expected that body weights in the tea/van-D animals would approach control levels if the study period were extended. It must be emphasized that the initial pretreatment glucose levels of both tea/van-D and D animals were very high (22.5 mmol/L). Thus, even extreme hyperglycemia was controlled well with vanadate/tea.

The mechanism by which vanadate lowers blood glucose levels is still not completely understood.[24,36-41] A mechanism proposed by Malabu et al suggested that appetite suppression alone produced the hypoglycemic effect.[41] However, McNeill et al disproved this postulate.[42] A more plausible possibility concerns the effects of vanadate on plasma insulin concentration or insulin action. The significantly increased plasma insulin levels found in the tea/van-D rats versus D animals provide evidence for either regeneration of β cells within the pancreatic islets or functional stimulation of those β cells not destroyed by STZ. Diabetic animals were not treated with vanadate/tea until 4 days after the STZ administration so the toxicity of STZ on the β cells could not have been affected by vanadate/tea. Electron microscopic analysis of pancreatic islets confirmed necrosis of a similar magnitude in β cells in both tea/van-D and D rats (data not shown). This would eliminate β-cell regeneration as a potential mechanism. Thus, it suggests that the remaining functional β cells are more active in the tea/van-D rats in order to control blood glucose. Some evidence exists to support this mechanism of action for vanadate.[24,36] In addition, vanadate may have an intracellular site of action in peripheral tissues. The intracellular phosphorylation process critical for the movement of the glucose transporter (GLUT 4) to the cell surface to induce glucose transport would be stimulated by phosphatase inhibition. Vanadate is a potent phosphatase inhibitor.[25-27] Thus, vanadate/tea may work through insulin receptor–independent and –dependent mechanisms to control blood sugar levels.

In summary, these results demonstrate that vanadate/tea is a potent hypoglycemic agent effective both acutely, and more importantly, chronically. Notably, this compound acts in the absence of any detectable side effects. The potential benefits offered by a long-lasting, oral route of administration cannot be underestimated considering the millions of insulin-dependent diabetic patients that currently require intramuscular injections on a daily basis.

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