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Short-term bioaccumulation of vanadium when ingested with a tea decoction in streptozotocin-induced diabetic rats

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Abstract

Sodium orthovanadate suspended in a lichee black tea decoction effectively regulates blood glucose levels in rats with insulin-dependent, streptozotocin (STZ)-induced diabetes. The primary advantage of vanadate delivery with the tea decoction over conventional systems that use water suspensions of vanadate is a significant reduction in the toxic side effects of vanadate. It is unknown if the tea alters the bioavailability of vanadate. Male Sprague-Dawley rats were administered an intravenous injection of STZ to induce diabetes. Four days later, the diabetic rats were treated by oral gavage with 40 mg of Na-orthovanadate suspended in double-distilled, deionized water (V/H_2O), tea/vanadate (TV) decoction, or were treated with the tea decoction alone. Vanadium concentrations were measured in blood and various tissues at 1 to 24 hours posttreatment using graphite furnace atomic absorption spectrophotometry. With the exception of bone, maximal vanadium concentration in plasma and tissue samples were observed 2 hours after ingestion, but steadily decreased after that. Plasma vanadium levels continued to decrease until 16 hours. In contrast, vanadium steadily accumulated in bone over the 24-hour period. Overall, rats treated with V/H_2O contained similar or significantly higher concentrations of vanadium in all tissues compared with TV treatment. The pattern of vanadium accumulation was also similar over time in both treatment groups. Vanadium levels were highest in bone > kidney > liver > pancreas > lung > heart > muscle > brain in both TV- and V/H_2O-treated animals. This study demonstrates that the accumulation of vanadium in diabetic rats is reduced when coadministered with a black tea decoction in comparison to administration of vanadium in water. However, this effect is unlikely to be of a magnitude to explain the full capacity of TV to reduce the toxic side effects of vanadate.

1. Introduction

Diabetes is the seventh leading cause of death in Canada and the United States [1]. Insulin and lifestyle changes help to delay the complications of diabetes, but to date, an ideal medication to prevent the deterioration of patients with diabetes is unavailable. In 1985, it was discovered that vanadium, a transition element, mimicked insulin and lowered diabetic glucose levels in vivo [2]. Vanadium is normally found in the body at microgram concentrations [3]. Vanadium is a potent phosphatase inhibitor and affects a multitude of enzymatic processes [4-6]. However, the effect of an accumulation of vanadate in organic tissue is uncertain. Large increases in intracellular and biologic stores of vanadium may cause metabolic derangements. Na-metavanadate, for example, has been found at high concentrations to cause structural changes to renal, splenic, and pulmonary tissue [7]. Severe vanadium toxicity, therefore, has delayed its use as a clinical therapy for diabetes [8,9].

A novel method of administering vanadate to diabetic rats has been developed that has shown excellent promise. Instead of suspending the vanadate in water as is used conventionally, it has been suspended in a lichee black tea decoction [10,11]. Insulin-deficient and insulin-resistant diabetic rats treated with this compound have been effectively maintained at nondiabetic glucose levels for up to 3 months with either reduced toxicity, or no side effects or toxicity [10,11]. The mechanism whereby the tea decoction

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protects against the toxic side effects of vanadate is unclear. Because vanadium toxicity to organ function within the body appears to be related to its accumulation within the relevant tissue, it is possible that the tea decoction is altering vanadate bioavailability. Therefore, we have analyzed the short-term accumulation of vanadium in tissue and plasma with tea/vanadate (TV) treatment in comparison to a conventional water-suspended vanadate delivery regimen.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 175 to 200 g were offered free access to both food (Prolab diet 5P00, PMI Nutrition International, Richmond, IN) and water. A 12-hour light-dark cycle was used with 6:00 AM to 6:00 PM as the light cycle. The animals were maintained at 20°C with 50% humidity. 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. Selected organs were surgically excised and stored at −80°C for future analysis.

2.2. Insulin-dependent diabetic model

Rats were lightly anesthetized, and tail vein injections of streptozotocin (STZ) (55 mg/kg body weight) were used to induce diabetes, as described previously [10-13]. Control animals received an injection of buffered vehicle alone. Animals were allowed to adjust to their diabetic state for 4 days before treatment. Four days post-STZ administration, all animal blood glucose levels were assessed using a Bayer glucometer elite testing system (Bayer, Etobicoke, ON, Canada) from 9:00 to 11:00 AM. A distal tail snip generated the 5-μL quantity of blood necessary for analysis. Subsequent glucose samples were obtained by removing the scab formed on the tail.

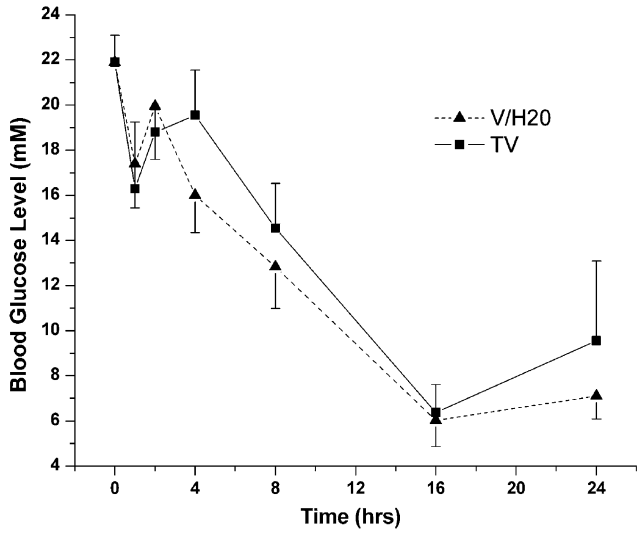

Fig. 1. Short-term effects of V/H_2O and TV treatment on blood glucose levels in STZ-induced diabetic rats. Each point represents mean ± SE (n = 6 in each treatment group).

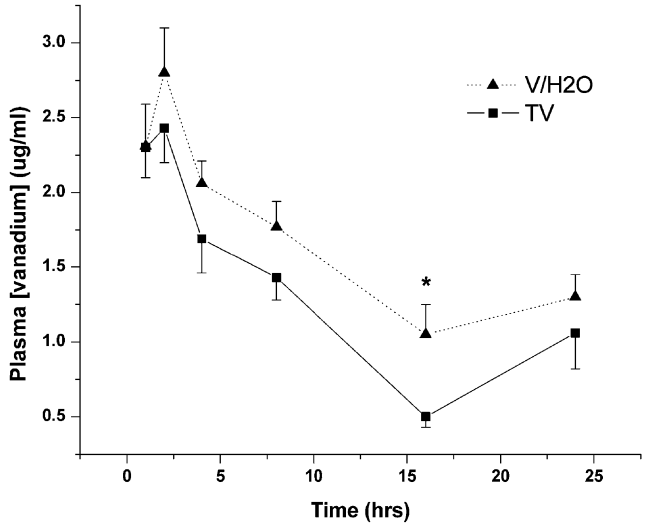

Fig. 2. Plasma vanadium levels in diabetic rats treated with a single dose of V/H_2O or TV. Each point represents mean ± SE (n = 6). *P < .05.

2.3. Treatment of diabetes

A lichee black tea decoction was made as described in detail [10,11]. Sodium orthovanadate (Sigma, St Louis, MO) was added to the decoction at a concentration of 20 mg vanadate per milliliter of tea decoction. This decoction was stored in the darkness at room temperature for 5 hours before use. A water/vanadate preparation was also used to treat the diabetic animals. This was prepared using the same methodology as for the TV suspension except replacing the tea decoction with deionized water. At 4:00 pm, all animals were orally gavaged with the appropriate treatment solution. Animal treatment groups included TV-treated diabetic (TVD) rats, Na-orthovanadate/double-distilled, deionized water–treated diabetic rats (V/H_2O), diabetic (D) rats, and nondiabetic (ND) rats. The latter 2 groups were treated with tea decoction alone (no vanadium added). Each animal received 2 mL of treatment solution, corresponding to a total vanadium dose of 40 mg.

2.4. Vanadium determinations: 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 stage used 4 mL concentrated HNO_3, the second used a combination of 2 mL HNO_3 and 30% H_2O_2, and the third and final stage used 2 mL HNO_3. All digestions were carried out at 130°C until complete drying of the sample was achieved. After the third drying, 1% HNO_3 was added to the digests and heated at 80°C for 1 hour. After cooling, the sample volume was measured and analyzed. Dilutions were made as required. Standard solutions of vanadium at varying concen-

trations 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 were made up in a 0.125-mol/L sodium citrate (Fisher Scientific, Ottawa, Ontario, Canada) solution containing 0.5% Triton X-100 (BDH Inc, Toronto, Ontario, Canada) for plasma analysis. Standard solutions from 0 to 100 μg/L were made up in 1% HNO_3 for tissue analysis.

Vanadium concentrations were measured using a polarized Zeeman graphite furnace atomic absorption (AA) spectrophotometer 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 10.0 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°C to 120°C ramped over 30 seconds with a hold time of 20 seconds and from 120°C to 450°C ramped over 25 seconds and held for 20 seconds. This was followed by an ashing step starting at 900°C to 1400°C ramped for 25 seconds and held for 20 seconds and finally atomization at 2850°C for 10 seconds. A 10-second cleaning step at 3000°C as well as a 5-second cool down were programmed for each run. Argon was used as the purge gas 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 upon relative correlations in peak absorbance. Instrument performance (result validity) was obtained through sample spikes, standard reruns, and for the tissue samples, digested blanks and digested bovine liver 1577b (standard reference material). The bovine 1577b Standard Reference Material contained a vanadium concentration of 0.123 μg/g. We ran these at 2 different concentrations at the start of every sample set and obtained a percentage of difference of 6.05% at 3.94 μg/L and a percentage of difference of 6.52% at

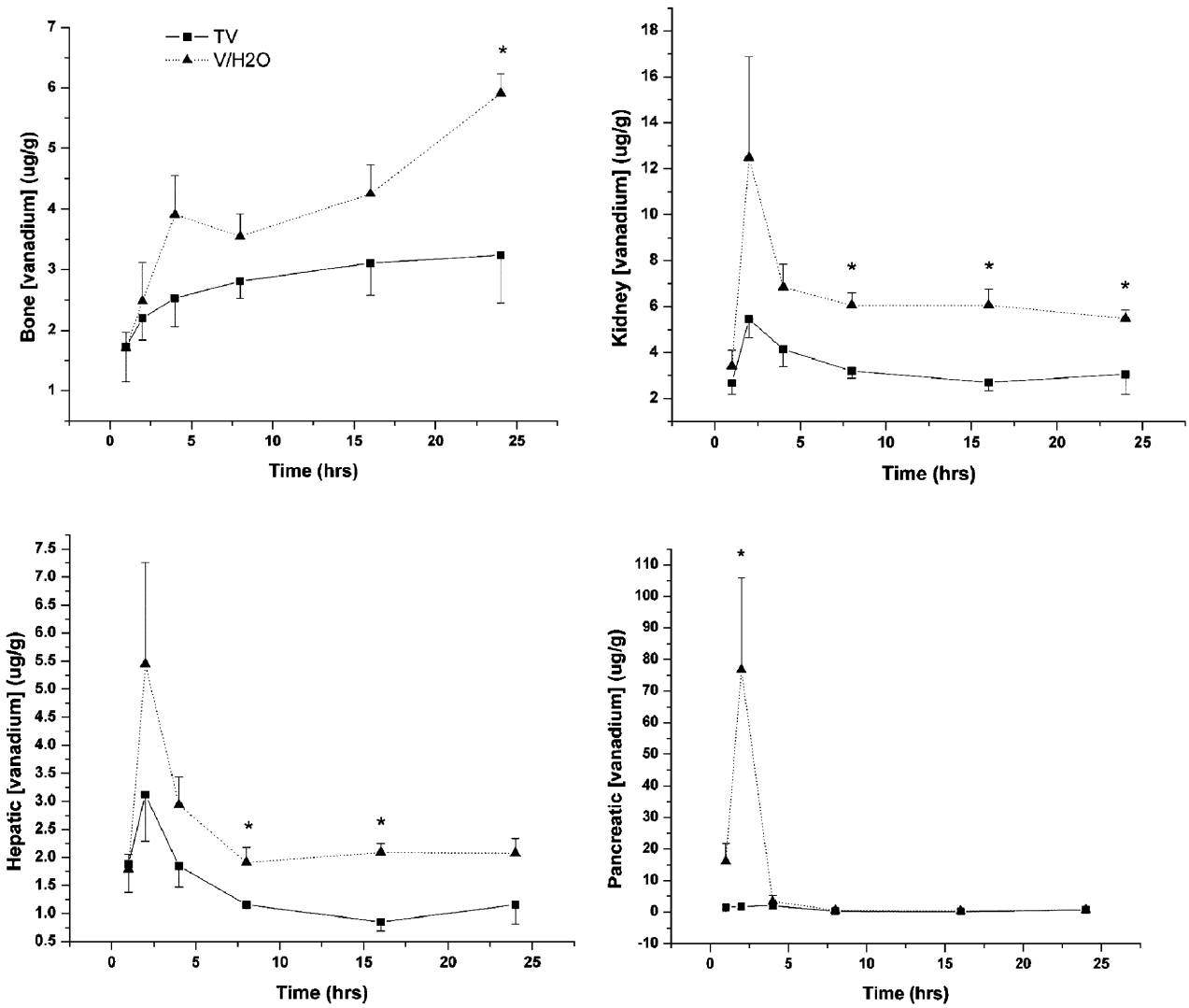

Fig. 3. Vanadium levels in bone (femur), kidneys, livers, and pancreas from diabetic rats treated with a single dose of V/H_2O or TV. Each point represents mean ± SE (n = 6). *$P < .05$.

5.90 μg/L. Recovery of spiked samples averaged 104.90%. The low-end detection limit of the instrument was 2.0 μg/L. All analyses were carried out in duplicate.

2.5. Statistical analysis

Statistical treatment of data was performed using a Students *t* test when only 2 groups were compared or an analysis of variance when more than 2 groups were compared. Results are reported as the mean ± SE. Statistical significance was determined at a *P* level of less than .05.

3. Results

The effect of both V/H_2O and TV on blood glucose levels is shown in Fig. 1. The initial blood glucose of the STZ-induced diabetic rats is approximately 22 mmol/L and identical in both the TV and V/H_2O treatment groups. This level is 4-fold higher than that of an ND Sprague-Dawley rat. After treatment, blood glucose levels fell within the first hour. During the second hour of posttreatment, blood glucose levels in both the V/H_2O- and TV-treated animals rose slightly. No statistically significant changes occurred over the next 2 hours. However, significant drops in blood glucose levels occurred during the 4- to 8-hour posttreatment interval in both vanadate-treated groups ($P < .01$). An even more marked hypoglycemic effect was achieved over the following 8-hour interval. Sixteen hours after vanadate treatment, the glucose levels in the V/H_2O and TVD rats were reduced to ND levels. The values at this point were 6.03 ± 1.16 and 6.38 ± 1.23 mmol/L, respectively, both significantly lower than the initial glucose levels ($P < .0001$). In the final 8 hours, glucose levels were elevated slightly to 7.11 ± 1.03 and 9.56 ± 3.53 mmol/L in V/H_2O and TVD rats, respectively.

Vanadium levels were measured in several organs and plasma to compare the uptake and storage of vanadate when delivered as V/H_2O and TV modalities. Plasma vanadium levels are shown in Fig. 2. All ND and D control animals had plasma vanadium levels lower than the detection limit of the AA spectrophotometer (2.00 μg/L). In contrast, the

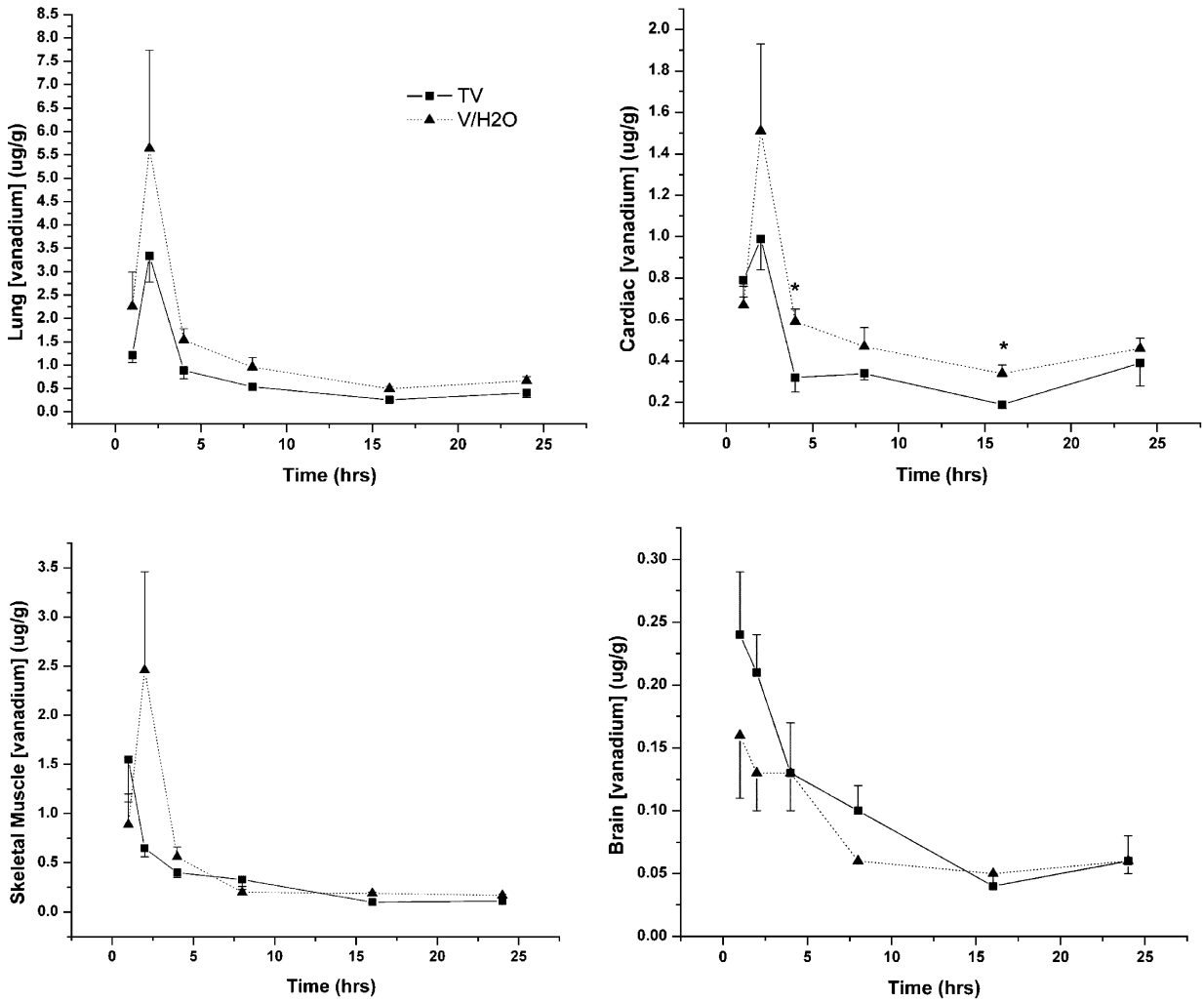

Fig. 4. Vanadium levels in lungs, hearts, skeletal muscle (gastrocnemius), and brains from diabetic rats treated with a single dose of V/H_2O or TV. Each point represents mean ± SE (n = 6). *$P < .05$.

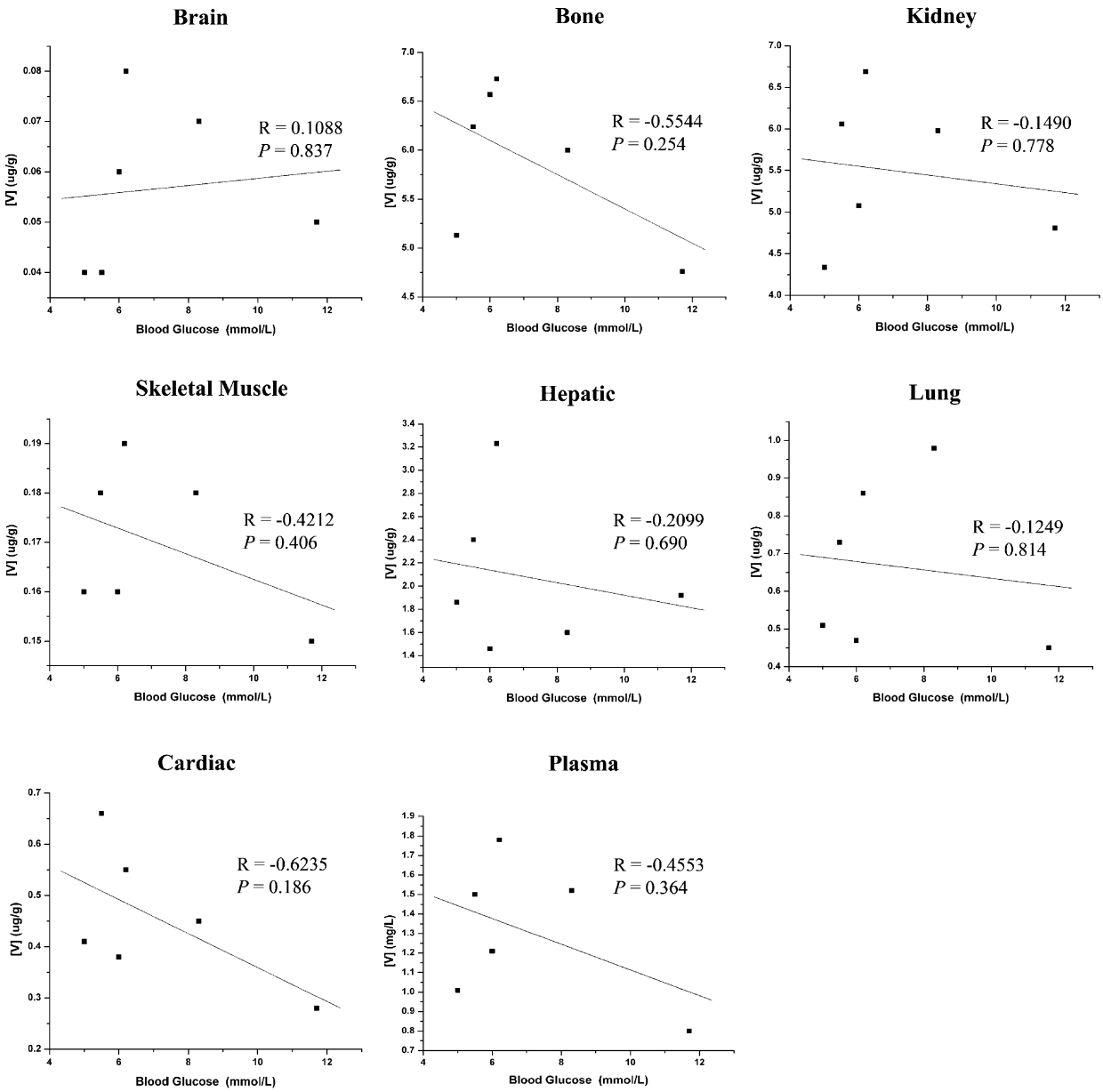

Fig. 5. Correlation of plasma and tissue levels of vanadium with plasma glucose levels in diabetic rats treated with a single dose of V/H_2O. Each point represents mean ± SE (n = 6).

vanadium-treated animals displayed detectable levels of vanadium at all time points. The highest plasma levels of vanadium were detected at the 1- and 2-hour time points (~2.5 mg/L) with decreasing concentrations up to 16 hours posttreatment. TV administration significantly reduced plasma vanadium concentration at the 16-hour time point compared with the V/H_2O treatment (0.50 ± 0.07 vs 1.05 ± 0.20 mg/L).

Vanadium concentrations were analyzed in 8 selected organs (Figs. 3 and 4). Vanadium concentrations were lower than the detection levels of the AA spectrophotometer in all organs of the ND or D animals. In the TV- and V/H_2O-treated animals, the highest concentrations were found in bone > kidney > liver > pancreas > lung > heart > muscle > brain. Leg bone measurements displayed a gradual increase in vanadium concentration over the 24-hour period. V/H_2O-treated animals retained significantly more vanadium than their TVD counterparts at the 24-hour time point (5.91 ± 0.32 vs 3.24 ± 0.79 μg/g, respectively). The increased accumulation of vanadium occurred solely over the 16- to 24-hour period (Fig. 3). Kidney accumulation was rapid and reached a maximum within the first 2 hours posttreatment. Levels remained stable from 4 to 24 hours with V/H_2O-treated animals accumulating significantly more vanadium than the TVD rats over all time points (Fig. 3). Temporal deposition of vanadium in liver paralleled that of the kidney, although levels of accumulation were lower (Fig. 3). Again, at 8 and

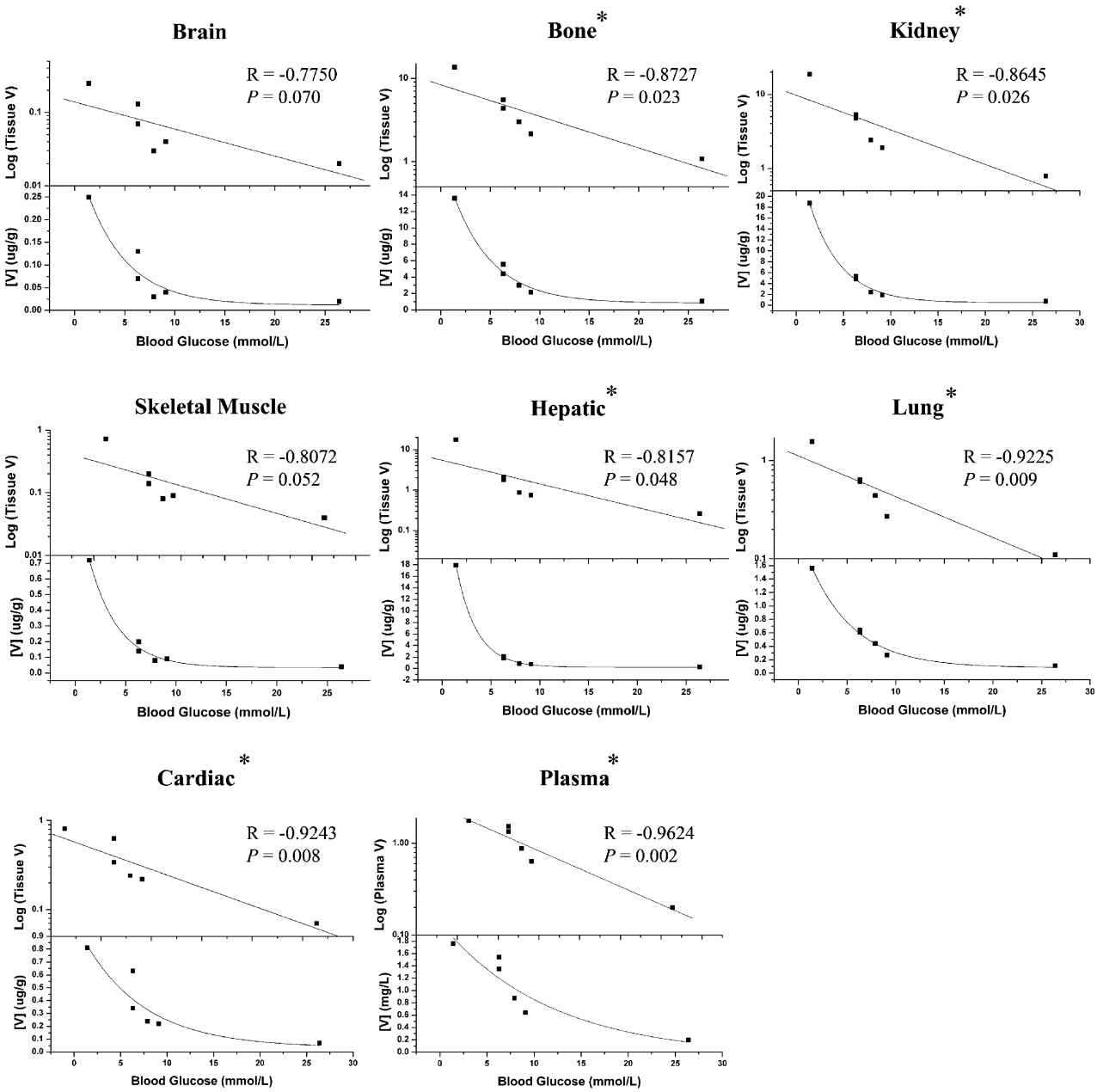

Fig. 6. Correlation of plasma and tissue levels of vanadium with plasma glucose levels in diabetic rats treated with a single dose of TV. Each point represents mean ± SE (n = 6). *$P < .05$.

16 hours, V/H_2O rats retained significantly more vanadium than the TVD rats. Vanadium concentrations in pancreatic samples displayed the largest variability (Fig. 3). Notwithstanding, V/H_2O-treated rats exhibited significantly higher levels of accumulation at 2 hours. All later time points showed no differences. Lung examination revealed similar levels of vanadium at all time points measured in the V/H_2O and TVD rats (Fig. 4). Levels peaked shortly after administration of the treatment solutions in both groups with 2-hour values of 5.64 ± 2.10 and 3.34 ± 0.56 μg/g in V/H_2O and TVD rats, respectively. By 4 hours and thereafter, levels remained at less than 1.0 μg/g. Significantly lower vanadium levels were found in hearts of rats receiving TV treatment than V/H_2O at both 4 and 16 hours (Fig. 4). Vanadium levels in TVD-treated rats reached plateau at approximately 0.3 μg/g after 4 hours. Muscle sections from the lower leg revealed little differences between treatment groups and minimal accumulation by 24 hours (Fig. 4). Levels in both groups were less than 0.20 μg/g by this time point. The lowest vanadium levels were found in brain (0.06 μg/g in V/H_2O and TVD rats) (Fig. 4). No differences at any time point were noted.

The plasma and tissue concentrations of vanadium were correlated with plasma glucose levels in all of the tissues.

This was done as a function of the delivery modality. In the case of V/H_2O-treated animals, there was a poor correlation of the 2 parameters (Fig. 5). This was true if the data were analyzed as a linear (Fig. 5) or a curvilinear (data not shown) correlation plot. However, there were significant correlations of plasma glucose levels and the plasma/tissue vanadium levels when the vanadium was delivered as TV (Fig. 6). This was particularly apparent if the data were analyzed as a curvilinear plot.

4. Discussion

The ability of vanadium to reduce hyperglycemia in animal models of diabetes was demonstrated approximately 3 decades ago [2]. The vanadium was suspended in water, and most subsequent studies continued this practice. The concentration of vanadium necessary to reduce hyperglycemia, however, was associated with some severe side effects including gastrointestinal toxicity and high mortality rates [7,10,11,14,15]. Instead of attempting to chemically modify the vanadium compound itself as has been used by many others [7-9,16,17], we have attempted the unconventional approach of suspending Na-orthovanadate in a tea decoction. Tea is known to have antidiarrhea effects and beneficial gastrointestinal properties [18]. This has resulted in successful lowering of blood glucose levels over long periods and minimal toxic side effects immediately or over several months of treatment [10,11].

In the present study, we examined the hypoglycemic effect of vanadium 24 hours after administration. As shown in Fig. 1, vanadium delivered as TV has an equivalent hypoglycemic action as the V/H_2O solution. Over the entire 24-hour time course, the diabetic rats treated with TV mimic the glucose levels of their V/H_2O-treated counterparts. Most importantly, at 16 hours posttreatment, the TV solution was capable of reducing the glycemic level of the diabetic animals to that of a nondiabetic rat (~6 mmol/L). Previously, it has been shown that long-term control of glucose is also achievable with TV treatment [10]. Diabetic rats treated with TV remained normoglycemic for an average of more than 3 weeks without further treatment, and these rats showed no signs of toxicity at 11 weeks of diabetic treatment [10].

It was hypothesized that TV would be able to control glucose without side effects by reducing the amount of vanadium remaining in the gastrointestinal tract lining during passage from the stomach to the sigmoid colon and therefore increasing its entry into the blood. Measurement of vanadium concentration in blood was therefore assessed (Fig. 2). However, this provides only an indirect assessment of vanadium clearance from the gastrointestinal area. Steady-state levels of plasma vanadium will also be influenced by clearance of vanadium from the blood into tissue compartments. Ingestion of tea is known to inhibit absorption of minerals and heavy metals from the gastrointestinal tract [19,20]. This would explain the reduced vanadium concentration in the blood at the 16-hour time point with the tea treatment. This surprisingly corresponds to the time point of maximal hypoglycemic action. Alternatively, tea has also been shown to alter mineral metabolism in bone [21] and brain [22], but both augmented and inhibition of absorption rates have been shown so it is difficult to determine the influence in the present study. The most likely hypothesis is that tea may influence absorption of minerals and heavy metals like vanadium from the gastrointestinal tract [19,20].

The biologic effects of vanadium are still unknown. Normal levels of vanadium in animals are very low (10-100 μmol/L). With the doses of vanadium used in this study, concerns about the possible toxicity to different organ systems are valid [14,15]. Tea lowered the amount of vanadium in blood, bone, kidney, liver, pancreas, and heart. No significantly increased vanadium levels were found in any organ at any time point with tea treatment in comparison to when the vanadium was administered in water. The potential for tea to reduce vanadium uptake over longer periods of treatment may therefore be a mechanism to reduce toxicity. Dai and colleagues [23,24] have shown that over a 1-year treatment period with vanadium in drinking water, accumulation occurred in a similar fashion to that in this study (bone > kidney > liver). It must be mentioned that Dai et al [23,24] found no organ dysfunction with these vanadium levels. Therefore, because TV significantly reduced vanadium levels in 5 of 8 organs vs V/H_2O after 1 treatment, the potential benefits of TV treatment over much longer treatment periods may be even more impressive. Indeed, this may explain the lack of organ toxicity observed in TVD animals when the compound was administered over a 3-month period [10]. Conversely, this does not explain the lack of toxicity in the gastrointestinal tract. It is possible that the vanadate is being cleared from the gut more quickly when presented with tea.

Alternatively, the complex of tea with vanadate may prevent the vanadate from inducing the diarrhea through unspecified mechanisms despite its presence in the gastrointestinal tract. One possibility involves the polyphenol content of tea. Polyphenols in tea such as epigallocatechin gallate and epicatechin gallate [25-27] have potent antioxidant action and chelating properties that may be involved in the effects observed in the present study. These properties of tea have been shown elsewhere to protect the body from a variety of disease conditions. Alternatively, the tannin content of tea is known to alter the absorption of minerals in the body [19]. Both tannins and polyphenols represent plausible components within tea to generate the effects observed in this study. However, it is important to note that chromatographic analyses of tea reveal a complex composition with hundreds of separate peaks that may ultimately play some role, synergistically or otherwise, in the effects observed in the present study.

In summary, the delivery of black tea with vanadate results in a lower accumulation of vanadate in most tissues

and the blood than when the vanadate is delivered in a conventional water vehicle. Despite this, TV effectively lowered blood glucose levels in the diabetic rats over a 24-hour period immediately after ingestion. As such, it appears to be an effective antidiabetic therapy. The lowered accumulation of vanadate in tissues may also limit toxicity over longer treatment periods [7,15]. Overall, the glycemic efficacy, the toxicity profile reported elsewhere and here combined with the short-term bioaccumulation profile demonstrated in this study would support the continued study of TV as an effective agent for the long-term treatment of diabetes mellitus.

Acknowledgment

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References

[1] Beamish Re, Dhalla NS, Pierce GN. Heart dysfunction in diabetes. Boca Raton (Fla): CRC Press; 1988.
[2] Heyliger CE, Tahiliani AG, McNeill JH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 1985;227:1474-7.
[3] Bryne AR, Kosta L. Vanadium in foods and in human body fluid and tissues. Sci Total Environ 1978;10:17-30.
[4] Shisheva A, Ikonomov O, Shechter Y. The protein tyrosine phosphatase inhibitor, pervanadate, is a powerful antidiabetic agent in streptozotocin-treated diabetic rats. Endocrinology 1994; 134:507-10.
[5] Swarup G, Cohen S, Garbers D. Inhibition of membrane phosphotyrosyl protein phosphatase activity by vanadate. Biochem Biophys Res Commun 1982;107:1104-9.
[6] Tamura S, Brown TA, Whipple JH, Fumjmita-yamaguchi J, Dubler RE, Cheng K, et al. A novel mechanism of the insulin-like effects of vanadate on glycogen synthase in rat adipocytes. J Biol Chem 1984;259:6650-8.
[7] Domingo JL, Gomez M, Llobet JM, Corbella J, Keen CL. Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used. Toxicology 1991;66:279-87.
[8] Boden G, Chen X, Ruiz J, van Rossum GD, Turco S. Effects of vanadyl sulfate on carbohydrate and lipid metabolism in patients with non–insulin-dependent diabetes mellitus. Metabolism 1996; 45:1130-5.
[9] Goldfine AB, Patti ME, Zuberi L, Goldstein BJ, LeBlanc R, Landaker EJ, et al. Metabolic effects of vanadyl sulfate in humans with non-insulin-dependent diabetes mellitus: in vivo and in vitro studies. Metabolism 2000;49:400-10.
[10] Clark TA, Heyliger CE, Edel AL, Goel DP, Pierce GN. The use of tea as a non-toxic modality to deliver vanadate to streptozotocin-induced diabetic rats. Metabolism 2004;53:1145-51.
[11] Clark TA, Edel AL, Heyliger CA, Pierce GN. Effective control of glycemic status and toxicity in Zucker diabetic fatty rats with an orally administered vanadate compound. Can J Physiol Pharmacol 2004; 82:888-94.
[12] Pierce GN, Ramjiawan B, Dhalla NS, Ferrari R. Na^{+}-H^{+} exchange in cardiac sarcolemmal vesicles isolated from diabetic rats. Am J Physiol 1990;258:H255-61.
[13] Pierce GN, Kutryk MJB, Dhalla NS. Alterations in calcium binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc Natl Acad Sci U S A 1983;80:5412-6.
[14] Srivastava AK. Anti-diabetic and toxic effects of vanadium compounds. Mol Cell Biochem 2000;206:177-82.
[15] Domingo JL. Vanadium and diabetes. What about vanadium toxicity? Mol Cell Biochem 2000;203:185-7.
[16] Yuen VG, Orvig C, Thompson KH, McNeill JH. Improvement in cardiac dysfunction in streptozotocin-induced diabetic rats following chronic oral administration of bis(maltolato)oxovanadium(IV). Can J Physiol Pharmacol 1993;71:270-6.
[17] Orvig C, Thompson KH, Battell M, McNeill JH. Vanadium compounds as insulin mimics. Met Ions Biol Syst 1995;31:575-94.
[18] Peirce A. The American Pharmaceutical Association practical guide to natural medicines. 1st ed. New York, NY: Stonesong Press; 1999.
[19] Chang MC, Bailey JW, Collins JL. Dietary tannins from cowpeas and tea transiently alter apparent calcium absorption but not absorption and utilization of protein in rats. J Nutr 1994;124:283-8.
[20] Heard MJ, Chamberlain AC, Sherlock JC. Uptake of lead by humans and effect of minerals and food. Sci Total Environ 1983;30:245-53.
[21] Das AK, Mukherjee M, Mitra C. Evidence for a prospective anti-osteoporosis effect of black tea (*Camellia sinensis*) extract in a bilaterally ovariectomized rat model. Asia Pac J Clin Nutr 2004; 13:210-6.
[22] Zeyuan D, Bingying T, Xiaolin L, Jinming H, Yifeng C. Effect of green tea and black tea on the metabolisms of mineral elements in old rats. Biol Trace Elem Res 1998;65:75-86.
[23] Dai S, Thompson KH, Vera E, McNeill JH. Toxicity studies on one-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulfate. Pharmacol Toxicol 1994;75:265-73.
[24] Dai S, McNeill JH. One-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulfate did not alter blood pressure or haematological indices. Pharmacol Toxicol 1994;74:110-5.
[25] Etus V, Altug T, Belce A, Ceylan S. Green tea polyphenol (−)-epigallocatechin gallate prevents oxidative damage on periventricular white matter of infantile rats with hydrocephalus. Tohoku J Exp Med 2003;200:203-9.
[26] Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG, Afana'ev IB. Influence of metal ions on flavonoid protection against asbestos-induced cell injury. Arch Biochem Biophys 2001; 385:129-37.
[27] Kostyuk VA, Potapovich AI, Vladykovskaya EN, Hiramatsu M. Protective effects of green tea catechins against asbestos-induced cell injury. Planta Med 2000;66:762-4.