Vanadate Improves Cardiac Function and Myocardial Energy Metabolism in Diabetic Rat Hearts

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SUMMARY

Vanadium mimicking the metabolic effects of insulin is known to decrease serum glucose levels and to influence glucose metabolism in diabetes mellitus. However, it is unclear whether vanadium ameliorates the metabolic disorder in diabetic hearts causing myocardial dysfunction. The purpose of this study was to assess the effects of vanadium on cardiac performance and energy metabolism in diabetic rat hearts. Four groups of Wistar rats were studied: untreated control rats (group C, n = 8), vanadate-treated rats (group V, n = 10), untreated diabetic rats (group DM, n = 9) induced by streptozotocin, and vanadate-treated diabetic rats (group DMV, n = 8). Vanadate-treated rats drank a 1.5 mM sodium orthovanadate (Na3VO4) solution during a 4 week diabetic condition. Hearts were perfused with Krebs-Henseleit buffer after the diabetic duration. After the maximum left ventricular dP/dt and cardiac efficiency were calculated, the myocardial contents of ATP and creatine phosphate (P-Cr) and myocardial energy metabolism were assessed by cytosolic phosphorylation potential. Peak positive and negative dP/dt, and cardiac efficiency decreased significantly in group DM compared with group C, while there were no significant differences between groups C and DMV. The myocardial contents of ATP (µmol/g wet heart) and P-Cr (µmol/g wet heart), and cytosolic phosphorylation potential (M-1) increased from 2.72 ± 0.46, 1.45 ± 0.58, and 3,530 ± 1,220 in group DM to 3.88 ± 0.76, 3.81 ± 1.36, and 11,200 ± 2,400 in group DMV, respectively. It is concluded that vanadium restored the production of high energy phosphates in the myocardium and improved myocardial dysfunction by regulating metabolic processes in diabetic rat hearts. (Jpn Heart J 2003; 44: 745-757)

Key words: Vanadate, Diabetes mellitus, Cardiac function, Energy metabolism

Vanadium, atomic number 23, is well known as an essential trace element and is present at varying amounts in various tissue compartments of the body. In particular, it is reported that the tissue content of vanadium is higher in the liver, kidney, and bones than in other organs. Vanadate, Na3VO4, expressed as the

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Received for publication October 22, 2002.
Revised and accepted December 26, 2002.

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+5 oxidized form of vanadium, is a phosphate analogue and has been proposed as an endogenous regulator of certain enzymes such as Na⁺, K⁺-ATPase, adenylate cyclase, and tyrosine phosphates.

Vanadium also exerts insulin-like actions on target tissues and many studies concerning its actions have been reported. In 1985, Heyliger, et al found that vanadium mimicked the metabolic effects of insulin, demonstrating that treatment with vanadium improved cardiac dysfunction in diabetic rat hearts by causing a decrease in blood glucose levels. However, exactly how vanadium regulates intracellular metabolic processes in diabetes mellitus has long been elusive and controversial. Furthermore, few studies have referred to the mechanism of metabolic change as the reason why vanadium ameliorates cardiac dysfunction in diabetic hearts.

The aim of the present study, therefore, was to evaluate the effects of vanadium on cardiac performance and to clarify their mechanisms concerning myocardial energy metabolism in diabetic rat hearts.

**METHODS**

**Treatment and maintenance of rats:** The study protocol was approved by the Committee on Animal Use of Kitasato University and the experiments were carried out in accordance with the National Institutes of Health guidelines for the use and care of animals. Thirty-five male Wistar rats weighing 300-350 g were used for the experiments and maintained in the animal house facility of Kitasato University at a constant temperature of 25°C with a 12-hr light and 12-hr dark cycle. The animals had access to rat feed (standard laboratory chow) and double distilled (DD) water ad libitum. The rats were randomly assigned to four groups as follows: untreated control group (group C, n = 8), vanadate-treated group (group V, n = 10), untreated diabetic group (group DM, n = 9) and vanadate-treated diabetic group (group DMV, n = 8).

In groups DM and DMV, diabetes mellitus was induced by an intraperitoneal injection of 40 mg streptozotocin (STZ) (194-08333, Wako Pure Chemical Industries Ltd., Osaka) per kg of body weight under ether anesthesia. The STZ solution was prepared by dissolving STZ in 0.091 M citrate buffer at pH 4.5. Rats with a serum glucose level greater than 350 mg/dL one week after the STZ injection were considered to have diabetes mellitus. The rats in groups C and DM continued to drink DD water for 4 weeks, while those in groups V and DMV were given a 1.5 mM solution of sodium orthovanadate (Na₃VO₄) for 4 weeks instead of DD water. A 1.5 mM solution of Na₃VO₄ dissolved in 0.5% sodium chloride served as the drinking water and was replaced with a freshly prepared solution everyday for groups V and DMV.
Experimental protocol: Four weeks after breeding, the rats were anaesthetized intraperitoneally with 40 mg of sodium pentobarbital per kg of body weight. After the heart was quickly excised and the adherent connective tissue removed in ice-cold buffer, the aorta of the heart was immediately cannulated for Langendorff perfusion. The heart was perfused with modified Krebs-Henseleit bicarbonate buffer in non-recirculating Langendorff mode, followed by working heart perfusion. The perfusate, which consisted of 143 mM Na⁺, 5.9 mM K⁺, 1.10 mM Ca²⁺, 0.52 mM Mg²⁺, 126 mM Cl⁻, 25 mM HCO₃⁻, 1.2 mM Pi, and 10 mM glucose, was adjusted to pH 7.40 by continuous oxygenation with a mixture of 95% O₂ and 5% CO₂, and its temperature was maintained at 38°C. A needle-tip pressure transducer (SPR-477, Millar Instruments Inc., Houston, TX, USA) was inserted into the left ventricular cavity through the apex of the heart to measure left ventricular pressure (LVP). Aortic pressure was measured by a pressure transducer (DTX, Disposable Transducer Kit, Spectramed Inc., Oxnard, CA, USA) connected to the aortic outflow tract with a fluid-filled catheter. Aortic pressure (AOP), heart rate (HR), LVP, and LV dP/dt were continuously monitored and recorded on polygraphs throughout the experimental period. Aortic flow (AOF) and coronary flow (CF) were measured volumetrically by collecting effluent from the aortic outflow line and from the pulmonary artery line, respectively. The oxygen pressure of perfusate from the aorta (PaO₂) and from the pulmonary artery (PvO₂) was analyzed after 15 minutes of stabilization in Langendorff mode with 60 mmHg of coronary perfusion pressure. After the initial measurements in the Langendorff mode, the perfusion mode was switched to working heart perfusion: the hearts performed work at varying AOP from 60 to 120 mmHg under a left atrial pressure of 10 cmH₂O. AOP, LVP, LV dP/dt, AOF, and CF were recorded and the oxygen pressure of the perfusate from the left atrium (PaO₂) and from the pulmonary artery (PvO₂) was analyzed in working heart perfusion (Figure 1). Finally, after being freeze-clamped using aluminum tongs equilibrated with liquid nitrogen, the isolated hearts were weighed and stored at -80°C until analysis of the metabolites.

Analytic techniques: Body weight was measured and then blood samples were collected from the tail vein to determine serum concentrations of glucose, Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺, inorganic phosphate (Pi), creatinine, insulin, and vanadium, when the rats were fasted for more than 6 hours before and after 4 weeks breeding.

Serum glucose was measured by the glucose oxidase method using an analyzer (DRI-CHEM 5500V, No. 9761208, Fuji Film, Tokyo) and the serum level of insulin was determined by immunoradiometric assay. We also measured the serum concentrations of Na⁺, K⁺, and Cl⁻ by the ion selective electrodes method, serum Ca²⁺ by the orthocresolphthalein complexone method,¹² serum Mg²⁺ by
the xylidyl blue method, serum Pi by the molybdic acid method, and serum creatinine by the Jaffe reaction. The serum concentration of vanadium was measured by atomic absorption spectrometry in which the lowest limit of detection was 0.02 ng/mL.

**Myocardial energy metabolism calculations:** Cardiac work, myocardial O\textsubscript{2} consumption (VO\textsubscript{2}), coronary resistance (CR), and cardiac efficiency were calculated using the values of AOF, CF, HR, AOP, PaO\textsubscript{2}, and PvO\textsubscript{2} as shown in Table I-1. The VO\textsubscript{2} in Langendorff perfusion was defined as the VO\textsubscript{2} of basal metabolism, which was measured without external work. Cardiac efficiency was calculated using VO\textsubscript{2} for external work in which the VO\textsubscript{2} of basal metabolism was excluded.

The myocardium freeze-clamped for assay was extracted with 3.6\% perchloric acid and neutralized, followed by enzymatical measurement of adenosine triphosphate (ATP), creatine phosphate (P-Cr), creatine (Cr), pyruvate (Pyr), lactate (Lac), dihydroxyacetone phosphate, and 3-phosphoglycerate. The Lac/Pyr ratio, the myocardial contents of adenosine diphosphate (ADP) and Pi, [ATP]/[ADP] ratio, cytosolic redox state ([NAD\textsuperscript{+}]/[NADH]) and cytosolic phosphorylation potential, (cytosolic [ATP]/[ADP]/[Pi]), based on the K\textsubscript{G+G}, K\textsubscript{LDH}, K\textsubscript{TPI} and K\textsubscript{CK} equilibrium constants, were calculated as shown in Table I-2.

**Statistical methods:** All values are expressed as the mean ± standard deviation (SD) and the myocardial metabolites are indicated as the content per g wet weight of myocardium. Differences among values obtained from each group were examined by ANOVA. Differences were considered to be statistically significant if the *P* value was less than 0.05.
RESULTS

Figure 2 shows the changes in serum glucose and body weight before and 4 weeks after vanadate treatment in the four groups. The serum concentrations of glucose (mg/dL) before treatment were 175 ± 14, 179 ± 26, 506 ± 46, and 511 ± 57 in groups C, V, DM, and DMV, respectively, and showed no significant differences between groups C and V, or between DM and DMV. Though there were no significant differences between the glucose levels before and after treatment in groups C and V, glucose levels after treatment increased significantly to 565 ± 33 in group DM (P < 0.001) and decreased significantly to 381 ± 55 in group DMV (P < 0.001). The elevation of serum glucose in diabetic rats was significantly restrained after the treatment in group DMV compared with group DM (P < 0.001).
The mean body weight (g) of the rats decreased significantly from 326 ± 16 and 315 ± 13 before treatment to 235 ± 30 and 254 ± 22 after treatment in groups DM (**P < 0.001) and DMV (**P < 0.001), respectively, while there were no significant changes in body weight during the 4-week-diabetic period in groups C and V. Furthermore, body weight was greater in group DMV than in group DM after the 4-week-diabetic period (**P < 0.001), although it decreased significantly in groups DM and DMV compared with group C (**P < 0.001 and **P < 0.001, respectively).

Table II shows the heart weights and serum concentrations of electrolytes 4 weeks after the treatment with vanadate. The ratio of heart weight to body weight was significantly higher in group DM than in groups C and DMV (**P < 0.001 and **P < 0.001, respectively). The serum concentration of insulin decreased significantly by 20% of the control value in group DM (**P < 0.001), by 25% in group DMV (**P < 0.001), and also by 60% in group V (**P < 0.001), whereas there was no significant difference in the insulin level between groups DM and DMV. The serum concentrations of Na⁺ and Cl⁻ were significantly lower in group DM than in groups C (**P < 0.001 and **P < 0.001, respectively) and DMV (**P < 0.001 and **P < 0.001, respectively), while there were no significant differences in K⁺, Ca²⁺, Mg²⁺, Pi, or creatinine among the four groups. The serum concentration of vanadium was significantly higher in groups V and DMV than in group C (**P < 0.001
and $P < 0.001$, respectively), while there was no significant difference in serum vanadium between groups C and DM.

Figure 3 shows HR, CR, and VO$_2$ in Langendorff perfusion. The HR values (/min.) were 230 ± 34, 249 ± 27, 173 ± 42, and 187 ± 39 in groups C, V, DM, and DMV, respectively, and decreased significantly in groups DM and DMV compared with group C ($P < 0.001$ and $P < 0.01$, respectively). However, there were no significant differences in CR, and VO$_2$ as internal work, among the four groups.

Figure 4 presents peak positive dP/dt, peak negative dP/dt, and cardiac efficiency for external work in the working heart perfusion. Peak positive and peak negative dP/dt, and cardiac efficiency were significantly lower in group DM than those in group C ($P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). However, peak positive dP/dt and cardiac efficiency improved significantly in group DMV compared with those in group DM ($P < 0.05$ and $P < 0.05$, respectively).

Figure 5 shows the myocardial contents of Pyr, Lac, and the Lac/Pyr ratio. The myocardial contents of Pyr and Lac were significantly higher in group DMV than those in group C ($P < 0.001$ and $P < 0.01$, respectively). The Lac/Pyr ratio demonstrated a significant increase in group DM compared with that in groups C and DMV ($P < 0.001$ and $P < 0.001$, respectively).

The myocardial contents of ATP, P-Cr, and Cr are presented in Figure 6. The myocardial contents of ATP and P-Cr were significantly lower in group DM than those in group C ($P < 0.001$ and $P < 0.01$, respectively) and improved significantly in group DMV compared with those in group DM ($P < 0.001$ and $P < 0.001$, respectively).

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<th>Table II. Serum Concentrations of Electrolytes and Vanadium</th>
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<td>Group</td>
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<td>Heart weight (g)</td>
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<td>Heart/BW (%)</td>
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<td>Serum insulin (µU/mL)</td>
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<td>Vanadium (ng/mL)</td>
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Values are expressed as mean ± SD. $^*$P < 0.01 and $^*$*$P < 0.001; vs group C, $^*$*$P < 0.001; vs group DMC = group C; V = group V; DM = group DM; DMV = group DMV. BW = body weight; Pi = inorganic phosphate.
Furthermore, group DMV showed a significantly higher P-Cr value and lower Cr value than group C ($P < 0.05$ and $P < 0.001$, respectively).

Figure 7 demonstrates the cytosolic $[\text{NAD}^+] / [\text{NADH}]$ and cytosolic phosphorylation potential. Cytosolic $[\text{NAD}^+] / [\text{NADH}]$ was significantly higher in group DMV than that in group DM ($P < 0.05$). The cytosolic phosphorylation
potential was significantly lower in group DM than that in group C \( (P < 0.001) \) and improved significantly in group DMV compared with that in group DM \( (P < 0.001) \).

Figure 5. Graphs showing the myocardial contents of Pyr (left), Lac (middle), and its L/P ratio (right) in rat hearts. Values are expressed as mean ± SD. Pyr = pyruvate; Lac = lactate; L/P = lactate/pyruvate. C = group C; V = group V; DM = group DM; DMV = group DMV.

**\( P < 0.01 \) and ***\( P < 0.001 \); vs group C. ###\( P < 0.001 \); vs group DM.

Figure 6. Graphs showing the myocardial contents of ATP (left), P-Cr (middle) and Cr (right) in rat hearts. Values are expressed as mean ± SD. ATP = adenosine triphosphate; P-Cr = creatine phosphate; Cr = creatine; C = group C; V = group V; DM = group DM; DMV = group DMV.

*\( P < 0.05 \), **\( P < 0.01 \) and ***\( P < 0.001 \); vs group C. ###\( P < 0.001 \); vs group DM.
DISCUSSION

Diabetes mellitus is widely known to complicate the impairment of cardiac performance that is mediated by coronary artery diseases including microangiopathy. Furthermore, it was reported that the cardiac dysfunction is caused by a metabolic disorder of the myocardium which is unrelated to coronary circulatory disturbance. Several mechanisms underlying the cardiac dysfunction suggest that microangiopathy induces diffuse myocardial ischemia, causing resultant micronecrosis and fibrosis in the myocardium. In metabolic abnormalities related to insulin deficiency, glucose oxidation is suppressed by the lowered activities of enzymes in glycolysis, resulting in a decrease in Pyr production and the sequential reduction of ATP. In addition, it has also been suggested that alterations in contractile proteins and abnormalities in calcium transients induce myocardial dysfunction in diabetes mellitus.

To prepare the experimental animals reflecting diabetes mellitus, it is established that STZ provides a permanent diabetes equivalent to clinically type I diabetes mellitus, which causes β-cell necrosis selectively in the pancreas. In the present study, the diabetic rats used were bred for 4 weeks after the induction of diabetes mellitus with STZ. It is thought microangiopathy had little effect on the cardiac dysfunction in groups DM and DMV because the changes in the systemic vasculature were minimum in the 4 week-duration from the onset of diabetes mellitus. Therefore, the CF and CR in Langendorff perfusion did not vary sig-
significantly among the four groups, indicating no alteration of coronary vasculature even in the presence of diabetes mellitus.

On the other hand, several studies have demonstrated that the administration of vanadium mimics the effects of insulin and normalizes the serum concentration of glucose in diabetic rats.\(^6,7\) In contrast to these previous studies, it was insufficient to normalize serum glucose levels in vanadate treatment to STZ-induced diabetes mellitus in the present study. Despite the significant increase in serum vanadium, the unsatisfactory glucose lowering effect of vanadium may be due to the different methods for administering vanadium or to the form of its complex. However, the findings of the present study revealed that the prominent action of vanadium was to decrease the serum concentration of insulin in group V rats which had normal pancreas function.\(^6,27\) This finding suggests that the antihyperglycemic effect of vanadium is not derived from an increase in insulin secretion from the pancreas, but from activation of the sensitivity of the target organs to insulin.\(^6,27\)

The present study also showed clearly that vanadate treatment resulted in the improvement of myocardial dysfunction by regulating the metabolic process in STZ-induced diabetes mellitus, confirming previous findings.\(^27\) Moreover, the present findings clarified that vanadate increased the depressed cardiac efficiency for external work in diabetic rats to the normal limits shown in control rats. It has been reported that vanadate possesses a positive inotropic effect on the heart that is due to the inhibition of Na\(^+\), K\(^+\) ATPase activity.\(^1,4\) However, the present study could not demonstrate a positive inotropic effect for vanadate because there was no significant difference in peak positive dP/dt between groups C and V. It is suggested that the improvement of cardiac dysfunction in diabetes mellitus results not from a positive inotropic effect of vanadate, but rather amelioration of the metabolic disorder in diabetic hearts.

With respect to changes in myocardial energy metabolism, diabetic rat hearts showed a significant decrease in the myocardial contents of ATP and P-Cr compared with the control rats, which was similar to the findings of previous studies.\(^21-24\) The lack of insulin is known to inhibit enzymes involved in glycolysis in type I diabetes mellitus,\(^23\) in addition to elevating the level of intracellular glucose following hyperglycemia.\(^28\) However, the oral administration of vanadate significantly increased the myocardial contents of Pyr and Lac over the baseline values obtained from group C and inhibited decreases in ATP, P-Cr, and cytosolic phosphorylation potential in diabetic rat hearts. It is believed the glycolysis enzymes activated by vanadium induced the marked increases in Pyr and Lac in a situation of elevated intracellular glucose.\(^29\) This metabolic process was confirmed by the findings showing no significant changes in either the L/P ratio or cytosolic [NAD\(^+\)]/[NADH], and elevated myocardial ATP.
Few toxic effects of vanadium were observed in the present study because vanadate administration to the normal control rats did not alter the serum concentration of glucose, body weight, cardiac performance, or myocardial metabolism. These findings suggest the possible clinical application of vanadium to replenish insulin deficiency in diabetic patients.

Vanadate may restore the supply of high energy phosphates by regulating the suppressed metabolic processes in diabetic rat hearts, and consequently ameliorate the myocardial dysfunction along with the increase in cardiac efficiency. **Study limitations:** Since it is uncertain in the present study whether vanadium affects the efficiency of energy production in the TCA cycle, further studies pertaining to the effects of vanadium on mitochondrial function are required.

Some previous studies have proposed the clinical application of vanadium as a supplemental therapy for insulin in diabetes mellitus because vanadium brought about improvements in metabolic disorders such as heart failure, microangiopathy, neuropathy, and cataract which were mediated by the lack of insulin.8,9) However, it is necessary to clarify any toxic effects of vanadium before it is used therapeutically.30)

**ACKNOWLEDGMENTS**

This study was supported by an Academic Frontier Project grant from the Japanese Ministry of Education, Science, Sports and Culture to Kitasato University School of Medicine.

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EFFECTS OF VANADATE IN DIABETIC RAT HEARTS


