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Vanadium Inhibits Type 2 Diabetes Mellitus-Induced Aortic Ultrastructural Alterations Associated with the Inhibition of Dyslipidemia and Biomarkers of Inflammation in Rats

El Vanadio Inhibe las Alteraciones Ultraestructurales Aórticas Inducidas por la Diabetes Mellitus Tipo 2 Asociadas con la Inhibición de la Dislipidemia y los Biomarcadores de Inflamación en Ratas

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SUMMARY: The potential inhibitory effect of the insulin mimicking agent, vanadium on type 2 diabetes mellitus (T2DM)-induced alterations to the aorta ultrastructure associated with the suppression of dyslipedima and biomarkers of inflammation has not been investigated before. Therefore, we tested whether vanadium can protect against aortic injury induced secondary to T2DM possibly via the inhibition of blood lipid and inflammatory biomarkers. T2DM was induced in rats by a high-fat diet and streptozotocin (50 mg/kg), and the treatment group started vanadium treatment five days post diabetic induction and continued until being sacrificed at week 10. Using light and electron microscopy examinations, we observed in the model group substantial damage to the aorta tissue such as damaged endothelium, degenerative cellular changes with vacuolated cytoplasm and thickened internal elastic lamina that were substantially ameliorated by vanadium. Administration of vanadium to diabetic rats also significantly ($p<0.05$) reduced blood levels of glucose, hyperlipidemia and biomarkers of inflammation (TNF-a, IL-6). We conclude that vanadium protects against T2DM-induced aortic ultrastructural damage in rats, which is associated with the inhibition of blood sugar and lipid and inflammatory biomarkers.

KEY WORDS: Diabetes; Aortic injury; Inflammation; Vanadium; Rat model.

INTRODUCTION

An estimated 70 % of people with diabetes die of cardiovascular complications (Laakso, 2010). Obesity is a major public health problem particularly in industrial and wealthy countries, and it is estimated that there are 35 million obesity-related deaths worldwide per year (Lustig *et al*., 2012). Dyslipidemia is regarded as a risk factor for the development and progression of hypertension (Yoshimura *et al*., 2011) and vascular injury (Rafieian-Kopaei *et al*., 2014). Abdominal obesity is a criterion of the metabolic syndrome, also called pre-diabetes, which is a cluster of abnormalities characterized by insulin resistance, inflammation, oxidative stress, hypertension and dyslipidaemia which affects up to 25 % of the population over the age of 50, and carries increased risk of type-2 diabetes mellitus, cardiovascular disease, non-alcoholic fatty liver disease and cancer (Kopelman, 2000; Eckel *et al*., 2005; Grattagliano *et al*., 2008).

In obesity, adipose tissues produce proinflamma-tory adipokines such as tumor necrosis factor-alpha (TNF-a), leptin, interleukin (IL-6), monocyte chemoattractant protein-1, resistin , and plasminogen activator inhibitor-1(PAI-1), that counteract adiponectin's function and enhance obesity-related vascular disease (Ronti *et al*., 2006; Barazzoni *et al*., 2012). In addition, oxidative stress is now considered to play a key role in metabolic and vascular derangements with an imbalance arising from exaggerated production and reduced elimination of free radicals (Sena *et al*., 2017).

Vanadium is a transitional element that is widely dispersed in nature, and its oral administration has been reported to improve DM in humans (Morsy *et al*., 2011) and experimental animals (Sakurai, 2002). A variety of different mechanisms by which vanadium improved diabetes have

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been suggested, such as changing of insulin sensitivity in the liver, kidney, and other tissues (Fantus & Tsiani, 1998; Karmaker *et al*., 2007). However, the potential inhibition of the aortic ultrastructural damage by vanadium induced secondary to T2DM in rats is unknown. Therefore, we speculated that T2DM-induced aortic injury in a rat model of diabetes could be inhibited with vanadium.

MATERIAL AND METHOD

Chemicals. Streptozotocin was supplied by (Sigma Chemical Company, USA), vanadium and sodium thiopental were purchased from (Bio-Chem, Austria).

Animals. Male Sprague Dawley rats weighing 150-200 g were used for the experiments with the approval of Ethical Committee of the college of medicine, King Khalid University, Abha, Saudi Arabia. The animals were obtained from the animal house of the College of Medicine of King Khalid University, where they were fed with standard rat's pellets and allowed free access to water. They were housed at a controlled ambient temperature of 25 ± 2 °C and 50 ± 10 % relative humidity, with 12-h light/12-h dark cycles. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Experimental design. After a one week adaptation period, 24 rats were randomly allocated into 4 groups (n= 6) as follows: Control group (Control): rats were injected intraperitoneally (i.p.) once with citrate buffer only (0.1 M, pH 4.5); Vanadium group (Van): rats were injected i.p. with a buffer as the control group and received vanadyl sulfate of 0.64 mmol/kg weight freshly dissolved in 1 ml of distilled water daily through an esophageal tube (Yuen *et al*., 1995); Type 2 diabetic group (T2DM): rats received a high-fat diet (HFD) for 2 weeks followed by a single i.p. injection of streptozotocin (STZ), 50 mg/kg BW (Gruzewska *et al*., 2014); and vanadium-treated diabetic group (T2DM+Van): rats received the same dose of vanadium as the vanadium group after 5 days of induction of diabetes. DM was verified by measuring blood glucose through tail–neck blood sampling. Rats with non-fasting blood glucose level of ≥11.1 mmol/L after 5 days of STZ injection were considered to be diabetic (Kedziora-Kornatowska *et al*., 1998). The daily treatments for the animals were continued for 8 weeks.

Preparation of blood and tissues for analysis. After 8 weeks, blood samples were collected under anesthesia using 40 mg Kg-1 sodium thiopentone, i.p., and animals were then culled. Aorta tissues were collected and fixed in 2.5 % glutaraldehyde for scanning electron microscopy examinations, or in 10 % formal saline for light microscopy. Sera were separated and stored at -80°C for subsequent measurements of biochemical parameters.

Transmission electron microscopy (TEM). Small pieces of aortic tissue were removed and immediately fixed in 2.5 % glutaraldehyde for 24 hours and washed with phosphate buffer (0.1 M, PH 7.4). Post-fixation was made in 1 % osmium tetroxide buffered to PH 7.4 with 0.1 M phosphate buffer at 4 °C for 1-2 hours. The samples washed in phosphate buffer to remove excess fixative, dehydrated through ascending grades of ethanol followed by clearing in propylene oxide. The specimens were embedded in Araldite 502, to form gelatin capsules. Polymerization was obtained by placing the capsules at 60 °C. Semi-thin sections (~1 mm thick)were stained with toluidine blue for orientation and observation. Ultra-thin sections (100 nm) were prepared using ultra-microtome and picked up on uncoated copper grids. Following double staining with uranylacetate and lead citrate, three-to-five random micrographs for each section were examined and photographed using a JEM-1011-JEOL transmission electron microscope, Japan, at 80 Kv.

Histological examination. Aorta specimens were immediately fixed in 10 % formal saline for 24 hours. Paraffin blocks were prepared,and 5 mm thick sections were subjected to hematoxylin and eosin (H&E) stain to elucidate the status of aortic architecture and the structural changes.

Determination of serum biochemical parameters. Blood levels of glucose were determined colourimetrically using a Randox reagent kit (Sigma-Aldrich). Quantitative determination of chemicals was purchased as follow: Triglyceride (TG), Total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) (Sigma), Interleukin-6 (IL-6) (RAYBIOTECH INC, MFR. No ELR-IL-6-001), tumor necrosis factor alpha (TNF-α) (BIOTANG INC, Cat. No.R6365), levels were measured according to the manufacturer's instructions.

Statistical analysis. The data was expressed as a mean ± standard deviation (SD). Data was processed and analyzed using the Graphpad prizim (version 6). One-way ANOVA was done followed by Tukey's post hoc test. Pearson correlation statistical analysis was done for detection of a probable significance between two different parameters. Results were considered significant if $P \leq 0.05$.

RESULTS

Induction of diabetes and aortic injury in rats by HFD and STZ. Feeding the model group of rats with HFD for two weeks followed by a single injection of STZ (50 mg/kg body weight, i.p.) caused a sharp increase in blood sugar and lipids and abnormal changes in aorta tissue architecture (Fig. 1). Significant ($p<0.05$) high blood levels of glucose and TG in the model group (T2DM) compared to normal levels in the control group were observed (Figs. 1A-B). H&E stained aorta sections of the model group revealed substantial damage in the endothelium and degenerated smooth muscle cells with vacuolated cytoplasm (Fig. 1D) compared to a normal tissue architucture in aorta sections obtained from the control group (Fig. 1C).

Vanadium protects against T2DM-induced aortic injury. We investigated the effect of vanadium treatment for 8 weeks on the development of T2DM-induced aortic ultrastructural alterations using TEM. TEM images of the aortic wall layers, endothelium and smooth muscle are shown in Figure 2. As expected normal architecture of endothelium and vascular smooth muscle cells was present in the untreated (Fig. 2A) and vanadium treated (Fig. 2B) control groups. These showed the normal appearance of endothelial cells (Ed) resting on a clear basement membrane adjacent to the lumen (Lu) of the blood vessel. The image of smooth muscle layer showed regularly-arranged smooth muscle cells (SMC). Whereas, the tunica intima (endothelium) and tunica media (smooth

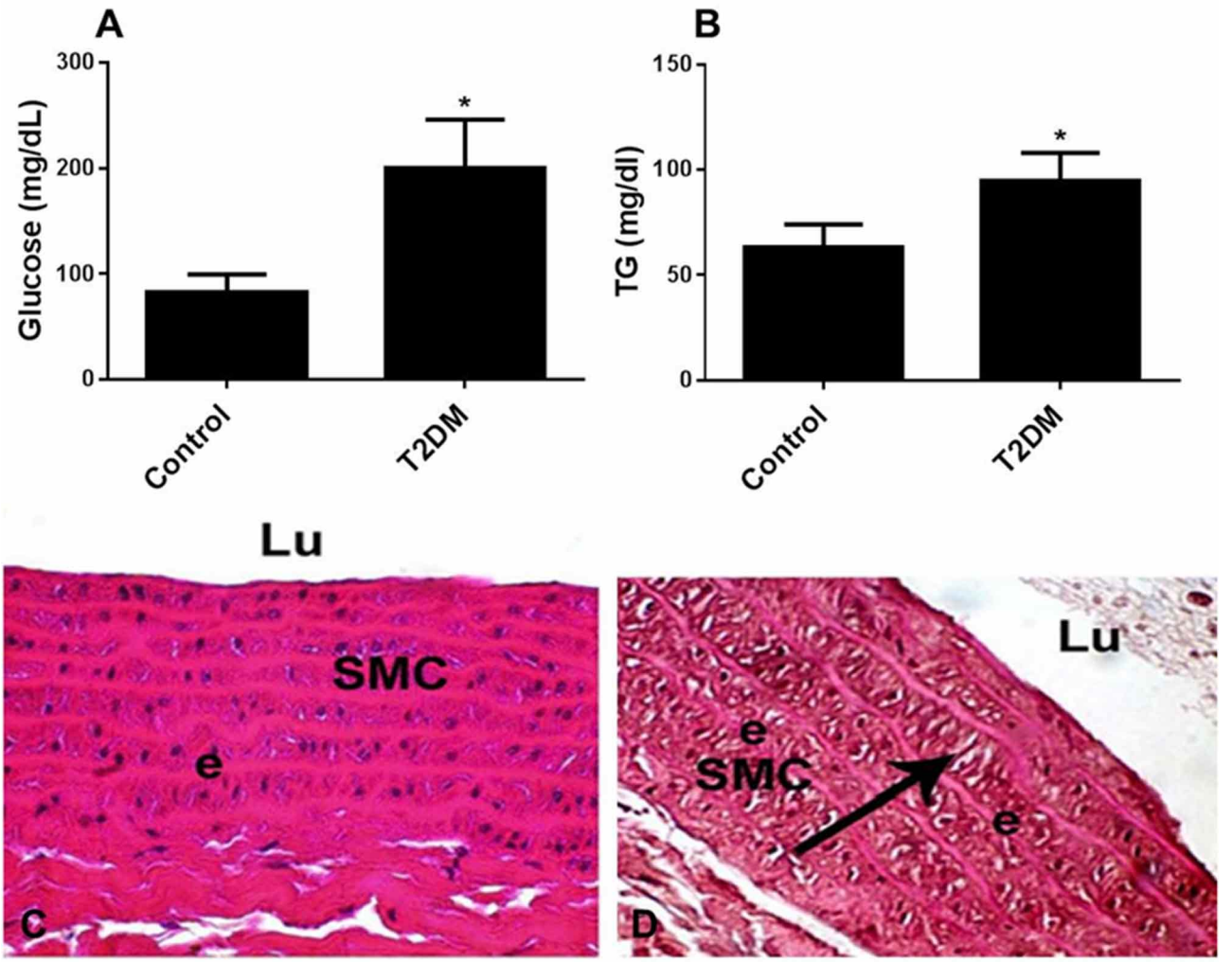

Fig. 1. Induction of T2DM and aorta injury in rats. Blood levels of glucose (A) and TG (B) were measured in the model group (T2DM) compared to the control group of rats (n=6 for each group). Results represent the mean (±SD), and experiments were performed in triplicate. *$p<0.05$ versus control. (C and D). H&E stained images (x400) of harvested tissues obtained from the aorta of model group (D) compared to the control group (C) rats are visualized using light microscopy. Note that arrow points to vacuolated cytoplasm. Abbreviations: SMC, smooth muscle cells; e, elastic lamina; Lu, lumen.

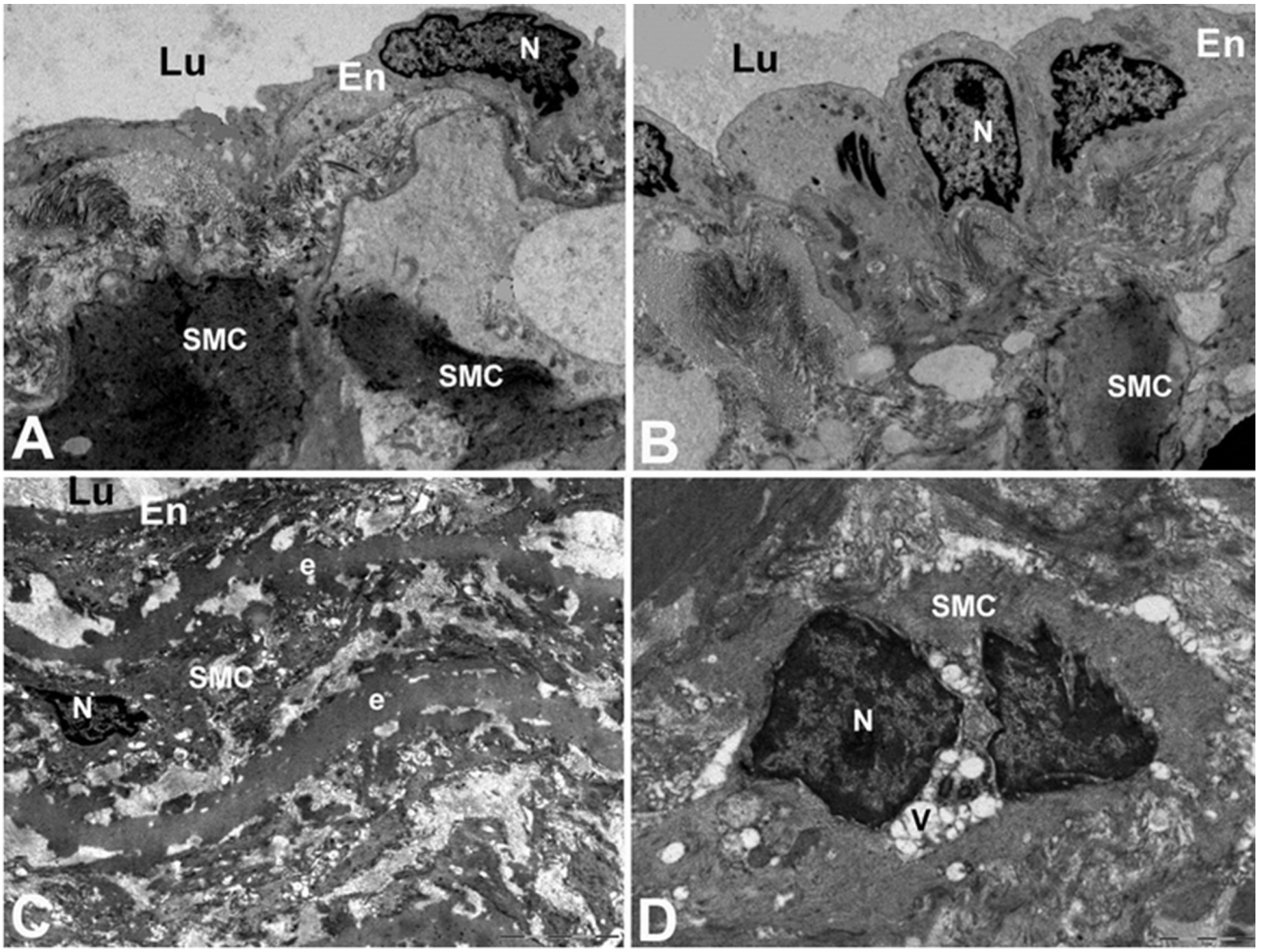

Fig. 2. Vanadium protects aorta ultrastructure in T2DM-induced aortic injury. TEM images (x5000) of the aortic wall layers tunica intima and tunica media obtained 8 weeks post diabetic induction. (A and B) Control groups. (C) Diabetic group. (D). Diabetic group treated with vanadium. Abbreviations: N, nucleus; SMC, smooth muscle cells; v, vacuoles; En, endothelial cell; Lu, lumen; e, elastic lamina.

muscle) of the aortic wall in T2DM rats (Fig. 2C) showed damaged endothelium and a degenerating smooth muscle cells and a relatively thickened internal elastic lamina (e). Vanadium treated group (T2DM+Van) demonstrated a protective effect to the aorta ultrastructure (Fig. 2D), however, vacuolated cytoplasm still be seen (arrow).

Vanadium reduces blood levels of glucose, TG, TC, and LDL-C in diabetic rats. As shown in Figure 3, administration of vanadium significantly ($p<0.05$) reduced T2DM-induced blood levels of glucose (Fig. 3A), TG (Fig. 3B), TC (Fig. 3C), and LDL-C (Fig. 3D). However, vanadium partially decreased blood glucose and LDL-C compared with the control group.

T2DM-induced biomarkers of inflammation are protected by vanadium. We measured blood levels of inflammatory biomarkers, TNF-α and IL-6 in all four rat groups. Diabetes caused a significant increase ($p<0.05$) in TNF-α (Fig. 4A) and IL-6 (Fig. 4B). Treatment with vanadium significantly ($p<0.05$) reduced these biomarkers. However, vanadium partially decreased TNF-α and IL-6 compared with the control group.

DISCUSSION

In this report, we investigated the aorta ultrastructure status in T2DM-induced aortic injury in a rat model of the disease in the presence and absence of vanadium. Also, our treatment protocol of vanadium was also used to assess blood levels of glucose, lipid, and inflammatory biomarkers. Here, we report the ability of vanadium to inhibit these biomarkers and ultrastructural alterations induced secondary to diabetes in a rat model of T2DM-induced aorta injury. Our H&E

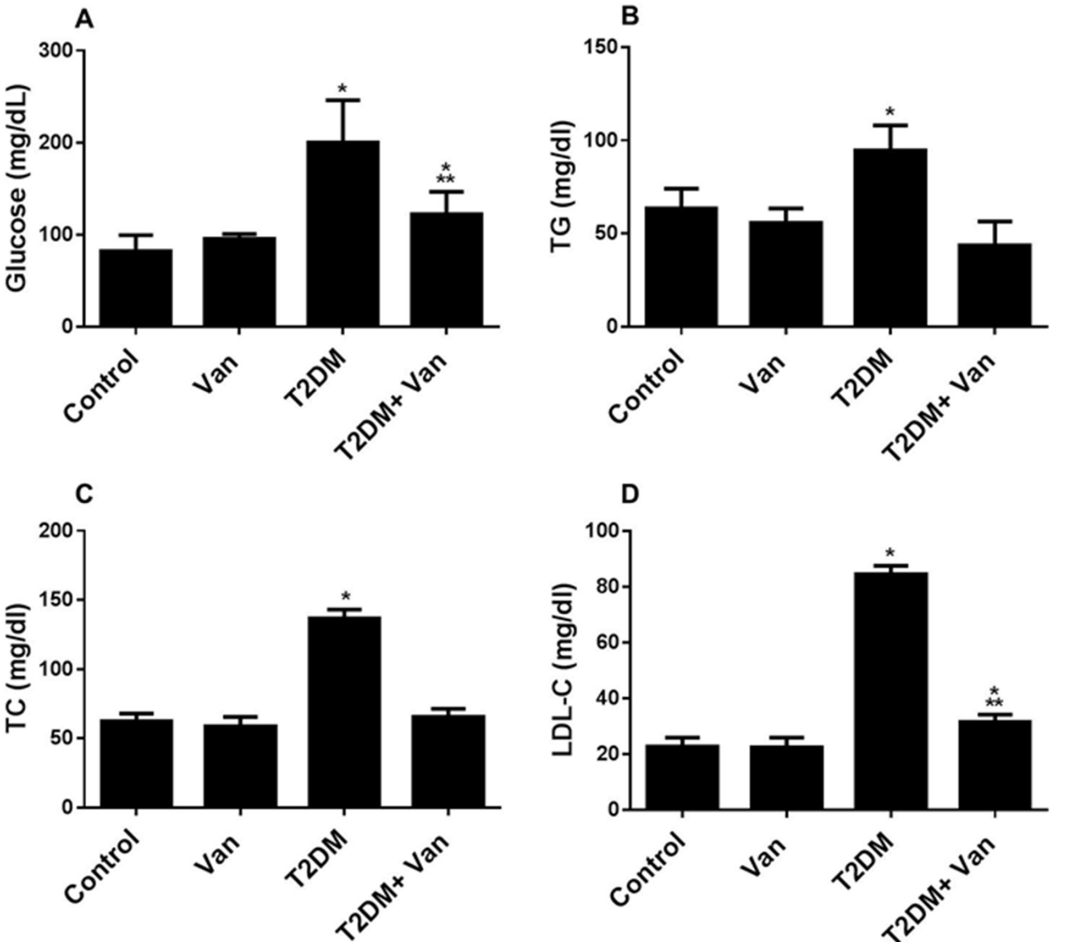

Fig. 3. Vanadium prevents hyperglycemia and lipidemia induced by T2DM. Blood glucose (A), TG (B), TC (C), and LDL-C (D) were measured 8 weeks post diabetic induction in 4 groups of rats; control, control vanadium (Van), diabetic (T2DM), and diabetic plus vanadium (T2DM+Van). Results represent the mean (±SD); n=6 for each group. Experiments were performed in triplicate. *p<0.05 versus control, **p<0.05 versus diabetic group, T2DM.

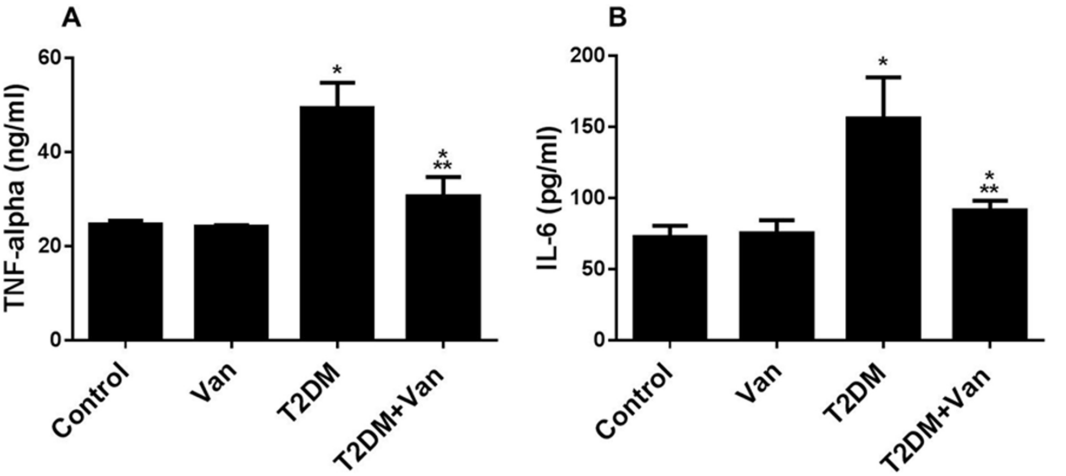

Fig. 4. Vanadium reduces circulating markers of inflammation induced by T2DM. Blood levels of TNF-α (A), and IL-6 (B) were measured 8 weeks post diabetic induction in 4 groups of rats; control, control vanadium (Van), diabetic (T2DM), and diabetic plus vanadium (T2DM+Van). Results represent the mean (±SD); n=6 for each group. Experiments were performed in triplicate. *p<0.05 versus control, **p<0.05 versus diabetic group, T2DM.

and TEM images supported our conclusions of aortic injury induced by T2DM after 8 weeks, which were treated with vanadium (Figs. 1 and 2 and data not shown). In addition, the data in Figures 3 and 4 show T2DM-induced up-regulation of circulating glucose, TG, TC, LDL-C, TNF-α, and IL-6, which were all reduced by vanadium treatment also supported our conclusions.

Elevated levels of inflammatory biomarkers like TNF-α and IL-6 are suggestive of active inflammatory diseases such as T1DM (Gomes, 2017), T2DM (Kasznicki *et al*., 2012), and vascular injury (Mu *et al*., 2015), and the aorta is a known target of both T1DM and T2DM in humans and animals (Turkbey *et al*., 2013; McCulloch *et al*., 2015; Hagensen *et al*., 2017). These reports are in agreement with our findings (Fig. 4), and the significant reduction in the levels of these markers upon treatment with vanadium (Fig. 4) might account for the observed improvement of the aorta ultrastructure (Fig. 2). In addition, a link between obesity and increased levels of oxidative stress and pro-inflammatory adipokines biomarkers such as leptin and IL-6 with vascular diseases was reported (Nseir *et al*., 2011), which are in agreement with our work that shows an association between the up-regulation of inflammation, and dyslipidemia and aortopathy (Figs. 1-4). Furthermore, our data that point to the reduction of blood sugar, triglycerides, total cholesterol and LDL-cholesterol by vanadium (Fig. 3) are in agreement with previous studies that demonstrated beneficial effects of vanadium in diabetes (Thompson & Orvig, 2006; Gruzewska *et al*.).

In summary, our data demonstrate that T2DM induced aortic ultrastructural alterations associated with hyperglycemia, dyslipidemia, and inflammation that were treated by vanadium.

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RESUMEN: El potencial efecto inhibidor del agente imitador de la insulina, el vanadio en las alteraciones inducidas por la diabetes mellitus tipo 2 (DM2) en la ultraestructura de la aorta, asociada con la supresión de dislipidemia y los biomarcadores de inflamación no se ha investigado anteriormente. El objetivo fue estudiar las propiedades del vanadio para proteger contra la lesión aórtica inducida a la DM2, a través de la inhibición de los lípidos sanguíneos y los biomarcadores inflamatorios. La DM2 fue inducida en ratas con una dieta alta en grasas y estreptozotocina (50 mg / kg), y el grupo de tratamiento fue sometido a un régimen continuo con vanadio, cinco días después de la inducción diabética hasta ser sacrificadas en la semana 10. Se utilizaron exámenes de luz y microscopía electrónica en el grupo modelo y se observó un daño sustancial al tejido de la aorta, como también en el endotelio; los cambios celulares degenerativos con citoplasma vacuolado y lámina elástica interna engrosada mejoró sustancialmente con vanadio. La administración de vanadio a ratas diabéticas también redujo significativamente ($p < 0,05$) los niveles sanguíneos de la glucosa, hiperlipidemia y los biomarcadores de inflamación (TNF-a, IL-6). En conclusión, el vanadio protege contra el daño ultraestructural aórtico inducido por T2DM en ratas, que es asociado con la inhibición del azúcar en la sangre y los biomarcadores de lípidos y de inflamatorios.

PALABRAS CLAVE: Diabetes; Lesión aórtica; Inflamación; Vanadio; Modelo de rata.

REFERENCES

Barazzoni, R.; Aleksova, A.; Armellini, I.; Cattin, M. R.; Zanetti, M.; Carriere, C.; Giacca, M.; Dore, F.; Guarnieri, G. & Sinagra, G. Adipokines, ghrelin and obesity-associated insulin resistance in nondiabetic patients with acute coronary syndrome. *Obesity (Silver Spring), 20(12)*:2348-53, 2012.

Eckel, R. H.; Grundy, S. M. & Zimmet, P. Z. The metabolic syndrome. *Lancet, 365(9468)*:1415-28, 2005.

Fantus, I. G. & Tsiani, E. Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. *Mol. Cell. Biochem., 182(1-2)*:109-19, 1998.

Gomes, K. B. IL-6 and type 1 diabetes mellitus: T cell responses and increase in IL-6 receptor surface expression. *Ann. Transl. Med., 5(1)*:16, 2017.

Grattagliano, I.; Palmieri, V. O.; Portincasa, P.; Moschetta, A. & Palasciano, G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J. Nutr. Biochem., 19(8)*:491-504, 2008.

Gruzewska, K.; Michno, A.; Pawelczyk, T. & Bielarczyk, H. Essentiality and toxicity of vanadium supplements in health and pathology. *J. Physiol. Pharmacol., 65(5)*:603-11, 2014.

Hagensen, M. K.; Mortensen, M. B.; Kjolby, M.; Stillits, N. L.; Steffensen, L. B. & Bentzon, J. F. Type 1 diabetes increases retention of low-density lipoprotein in the atherosclerosis-prone area of the murine aorta. *Atherosclerosis, 263*:7-14, 2017.

Karmaker, S.; Saha, T. K.; Yoshikawa, Y. & Sakurai, H. Amelioration of hyperglycemia and metabolic syndromes in type 2 diabetic KKA(y) mice by poly(gamma-glutamic acid)oxovanadium(IV) complex. *Chem. Med. Chem., 2(11)*:1607-12, 2007.

Kasznicki, J.; Kosmalski, M.; Sliwinska, A.; Mrowicka, M.; Stanczyk, M.; Majsterek, I. & Drzewoski, J. Evaluation of oxidative stress markers in pathogenesis of diabetic neuropathy. *Mol. Biol. Rep., 39(9)*:8669-78, 2012.

Kedziora-Kornatowska, K. Z.; Luciak, M.; Blaszczyk, J. & Pawlak, W. Effect of aminoguanidine on erythrocyte lipid peroxidation and activities of antioxidant enzymes in experimental diabetes. *Clin. Chem. Lab. Med., 36(10)*:771-5, 1998.

Kopelman, P. G. Obesity as a medical problem. *Nature, 404(6778)*:635-43, 2000.

Laakso, M. Cardiovascular disease in type 2 diabetes from population to man to mechanisms: the Kelly West Award Lecture 2008. *Diabetes Care, 33(2)*:442-9, 2010.

Lustig, R. H.; Schmidt, L. A. & Brindis, C. D. Public health: The toxic truth about sugar. *Nature, 482(7383)*:27-9, 2012.

McCulloch, M. A.; Mauras, N.; Canas, J. A.; Hossain, J.; Sikes, K. M.; Damaso, L. C.; Redheuil, A.; Ross, J. L. & Gidding, S. S. Magnetic resonance imaging measures of decreased aortic strain and distensibility are proportionate to insulin resistance in adolescents with type 1 diabetes mellitus. *Pediatr. Diabetes, 16(2)*:90-7, 2015.

Morsy, M. D.; Abdel-Razek, H. A. & Osman, O. M. Effect of vanadium on renal Na+,K+-ATPase activity in diabetic rats: a possible role of leptin. *J. Physiol. Biochem., 67(1)*:61-9, 2011.

Mu, W.; Chen, M.; Gong, Z.; Zheng, F. & Xing, Q. Expression of vascular cell adhesion molecule-1 in the aortic tissues of atherosclerotic patients and the associated clinical implications. *Exp. Ther. Med., 10(2)*:423-8, 2015.

Nseir, W.; Shalata, A.; Marmor, A. & Assy, N. Mechanisms linking nonalcoholic fatty liver disease with coronary artery disease. *Dig. Dis. Sci., 56(12)*:3439-49, 2011.

Rafieian-Kopaei, M.; Setorki, M.; Doudi, M.; Baradaran, A. & Nasri, H. Atherosclerosis: process, indicators, risk factors and new hopes. *Int. J. Prev. Med., 5(8)*:927-46, 2014.

Ronti, T.; Lupattelli, G. & Mannarino, E. The endocrine function of adipose tissue: an update. *Clin. Endocrinol. (Oxf.), 64(4)*:355-65, 2006.

Sakurai, H. A new concept: the use of vanadium complexes in the treatment of diabetes mellitus. *Chem. Rec., 2(4)*:237-48, 2002.

Sena, C. M.; Pereira, A.; Fernandes, R.; Letra, L. & Seiça, R. M. Adiponectin improves endothelial function in mesenteric arteries of rats fed a high-fat diet: role of perivascular adipose tissue. *Br. J. Pharmacol., 174(20)*:3514-26, 2017.

Thompson, K. H. & Orvig, C. Vanadium in diabetes: 100 years from Phase 0 to Phase I. *J. Inorg. Biochem., 100(12)*:1925-35, 2006.

Turkbey, E. B.; Redheuil, A.; Backlund, J. Y.; Small, A. C.; Cleary, P. A.; Lachin, J. M.; Lima, J. A.; Bluemke, D. A. & Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Aortic distensibility in type 1 diabetes. *Diabetes Care, 36(8)*:2380-7, 2013.

Yoshimura, N.; Muraki, S.; Oka, H.; Kawaguchi, H.; Nakamura, K. & Akune, T. Association of knee osteoarthritis with the accumulation of metabolic risk factors such as overweight, hypertension, dyslipidemia, and impaired glucose tolerance in Japanese men and women: the ROAD study. *J. Rheumatol., 38(5)*:921-30, 2011.

Yuen, V. G.; Orvig, C. & McNeill, J. H. Comparison of the glucose-lowering properties of vanadyl sulfate and bis(maltolato)oxovanadium(IV) following acute and chronic administration. *Can. J. Physiol. Pharmacol., 73(1)*:55-64, 1995.

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