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

Ismaeel Bin-Jaliah1; M. D. Morsy1,2; Bahjat Al-Ani1; Refaat A. Eid3 & Mohamed A. Haidara1,4


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 dyslipidemia 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-α, 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 proinflammatory adipokines such as tumor necrosis factor-alpha (TNF-α), 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
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 ≤ 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 architecture 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.
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.

**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.
El potencial efecto inhibidor del agente inhibitor de la insulina, el vanadio, en las alteraciones ultraestructurales aórticas inducidas por la diabetes mellitus tipo 2 (DM2) en ratas, es compatible con estudios anteriores que han reportado beneficios de la administración de vanadio en pacientes no diabéticos con síndrome metabólico (Nseir et al., 2017). Estos estudios concuerdan con nuestros hallazgos (Fig. 3) que sugieren modificaciones beneficiosas en la aorta de ratas diabéticas a la administración de vanadio (Fig. 4).

En conclusión, el vanadio protege contra el daño aórtico inducido por DM2 en ratas, lo que está asociado con la inhibición del aterosclerosis. El vanadio inhibe las alteraciones de lípidos y de inflamatorios. La administración de vanadio a ratas diabéticas también redujo significativamente (p <0,05) las niveles sanguíneos de la glucosa, hiperlipidemia y los biomarcadores de inflamación (TNF-α, IL-6). En conclusión, el vanadio protege contra el daño ultraestructural aórtico inducido por DM2 en ratas, que es asociado con la inhibición del aterosclerosis en la sangre y los biomarcadores de lípidos y de inflamatorios.

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

REFERENCES


Corresponding author:
Professor Mohamed A Haidara
Department of Physiology
College of Medicine
King Khalid University
Abha 61421
SAUDI ARABIA

E-mail : haidaram@hotmail.com

Received: 26-08-2019
Accepted: 13-09-2019