Insulin and vanadium protect against osteoarthritis development secondary to diabetes mellitus in rats

Abbas O. El Karib, Bahjat Al-Ani, Fahaid Al-Hashem, Mohammad Dallak, Ismaeel Bin-Jaliah, Basiouny El-Gamal, Salah O. Bashir, Refaat A. Eid & Mohamed A. Haidara

To cite this article: Abbas O. El Karib, Bahjat Al-Ani, Fahaid Al-Hashem, Mohammad Dallak, Ismaeel Bin-Jaliah, Basiouny El-Gamal, Salah O. Bashir, Refaat A. Eid & Mohamed A. Haidara (2016) Insulin and vanadium protect against osteoarthritis development secondary to diabetes mellitus in rats, Archives of Physiology and Biochemistry, 122:3, 148-154, DOI: 10.3109/13813455.2016.1159698

To link to this article: http://dx.doi.org/10.3109/13813455.2016.1159698
ORIGINAL ARTICLE

Insulin and vanadium protect against osteoarthritis development secondary to diabetes mellitus in rats

Abbas O. El Karib1, Bahjat Al-Ani1, Fahaid Al-Hashem1, Mohammad Dallak1, Ismaeel Bin-Jaliah1, Basiouny El-Gamal2, Salah O. Bashir1, Refaat A. Eid3, and Mohamed A. Haidara1,4

1Department of Physiology, 2Department of Clinical Biochemistry, and 3Department of Pathology, College of Medicine, King Khalid University, Abha, Saudi Arabia, and 4Department of Physiology, Kasr al-Aini Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract

Objective: Diabetic complications such as cardiovascular disease and osteoarthritis (OA) are among the common public health problems. The effect of insulin on OA secondary to diabetes has not been investigated before in animal models. Therefore, we sought to determine whether insulin and the insulin-mimicking agent, vanadium can protect from developing OA in diabetic rats. Methods: Type 1 diabetes mellitus (T1DM) was induced in Sprague–Dawley rats and treated with insulin and/or vanadium. Tissues harvested from the articular cartilage of the knee joint were examined by scanning electron microscopy, and blood samples were assayed for oxidative stress and inflammatory biomarkers. Results: Eight weeks following the induction of diabetes, a profound damage to the knee joint compared to the control non-diabetic group was observed. Treatment of diabetic rats with insulin and/or vanadium differentially protected from diabetes-induced cartilage damage and deteriorated fibrils of collagen fibers. The relative biological potencies were insulin + vanadium > insulin > vanadium. Furthermore, there was about 2- to 5-fold increase in TNF-α (from 31.02 ± 1.92 to 60.5 ± 1.18 pg/ml, p < 0.0001) and IL-6 (from 64.67 ± 8.16 to 338.0 ± 38.9 pg/ml, p < 0.0001) cytokines and free radicals measured as TBARS (from 3.21 ± 0.37 to 11.48 ± 1.5 μM, p < 0.0001) in the diabetic group, which was significantly reduced with insulin and or vanadium. Meanwhile, SOD decreased (from 17.79 ± 8.9 to 8.25 ± 0.29, p < 0.0001) cytokines and free radicals measured as TBARS (from 3.21 ± 0.37 to 11.48 ± 1.5 μM, p < 0.0001) in the diabetic group, which was significantly reduced with insulin and or vanadium. Meanwhile, SOD decreased (from 17.79 ± 8.9 to 8.25 ± 0.29, p < 0.0001) and was increased with insulin and vanadium. The relative potencies of the treating agents on inflammatory and oxidative stress biomarkers were insulin + vanadium > insulin > vanadium. Conclusion: The present study demonstrates that co-administration of insulin and vanadium to T1DM rats protect against diabetes-induced OA possibly by lowering biomarkers of inflammation and oxidative stress.

Introduction

An estimated 347 million people around the world suffer from diabetes, which is one of the leading global health care problems that claims the life of about 3.4 million every year (Danaei et al., 2011). Type 1 diabetes mellitus (T1DM) is believed to be caused by autoimmune antibodies targeting the β cells-producing insulin in the pancreas that leads to the loss of insulin production and hence the appearance of diabetes symptoms in children, commonly between the ages of 7–19 years (Atkinson et al., 2014; Narendran et al., 2005; Pugliese, 2004).

Osteoarthritis (OA) is a degenerative joint disease that involves degradation and destruction of the articular cartilage structure of the joint leading to pain, swelling and reduced joint movement; and there is increasing evidence to link metabolic disturbances such as diabetes to OA (Pottie et al., 2006, Van Manen et al., 2012). OA of the knee and/or hip is regarded as one of the most prevalent conditions leading to disability particularly in the elderly population (Grazio & Balen, 2009). Knee OA is more important, not only for its high prevalence rate compared with other types of OA, but also for its presentation at earlier age groups particularly in younger age groups of obese women (Bliddal & Christensen, 2009; Hayami, 2008).

Diabetic limited joint mobility syndrome is seen in 8–50% of T1DM patients. The lack of the bone anabolic actions of insulin and other pancreatic hormones could be the reason why T1DM affects the skeleton more severely than type 2 diabetes mellitus (T2DM) (Hamann et al., 2012; Janghorbani et al., 2007). In a study of patients with T2DM followed over a course of 20 years it was concluded that longstanding diabetes per se is detrimental for knee and hip joints, leading to progressive destruction and joint failure (Schett et al., 2013).

* These authors contributed equally to this work.
Correspondence: Professor Mohamed Haidara, Department of Physiology, College of Medicine, King Khalid University, Abha 61421, Saudi Arabia. Tel: 00966540733723. E-mail: haidaram@hotmail.com

Keywords

Diabetes, T1DM, osteoarthritis, insulin, vanadium, rat model

History

Received 28 November 2015
Revised 28 January 2016
Accepted 24 February 2016
Published online 23 March 2016
Proinflammatory biomarkers IL-6 and TNF-α are significant predictors of knee OA (Kou & Wu, 2014; Livshits et al., 2009) and OA cartilages from DM patients showed increased responsiveness to IL-1β-induced inflammation via oxidative stress and polyol pathway thus participate in the increased inflammation in OA (Laiguillon et al., 2015).

Vanadium is a trace mineral that is widely distributed in nature and has been reported to ameliorate DM in humans when administered orally (Gaede et al., 2008). The glucose-lowering effect of vanadium is attributed to the insulin-like function of the compound and its ability to enhance insulin activity and cellular bioavailability (Haidara et al., 2015; Karmaker et al., 2007). Vanadium induces its effect through insulin receptor-independent pathways since it has no effect in activating the insulin receptor tyrosine kinase, a key enzyme in insulin cell signalling (Shechter et al., 1995). However, vanadium exerts its effects through inhibition of protein tyrosine phosphatases, mainly PTEN and activation of cytosolic tyrosine kinases that augment and activate downstream insulin receptor cell signalling (Srivastava & Mehdi, 2005). Studies have shown that vanadyl sulphate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the streptozotocin (STZ) diabetic rat, the Zucker fatty rat and the Zucker diabetic fatty rat (Gruzewska et al., 2014).

Interestingly, neither the effect of insulin nor its mimicking agent, vanadium on diabetes-induced OA in animal models has been addressed before. Therefore, we were interested to study the potential protective effect of insulin and or vanadium against OA of the knee joint secondary to T1DM, and to see if vanadium adjuvant treatment with insulin can augment the proposed protection. To answer these questions, we developed an animal model of T1DM-induced OA in Sprague–Dawley rats and used the model to evaluate the effect of insulin and vanadium on OA.

Methods

Animals

The experiments were performed on 36 male Sprague–Dawley rats of 10 weeks old and weighting 200–250 g. The rats were fed with standard laboratory diets, given water ad libitum and maintained under laboratory conditions of temperature (22 ± 3°C), with 12-h light and 12-h dark cycle. All experimental procedures involving the handling and treatment of animals were approved by the Research Ethical Committee of King Khalid University (Abha, KSA) and were conducted in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals.

Animal protocol

After 1-week adaptations, animals were classified and randomly allocated into 6 groups (n = 6) as follows: control group (Control): non-diabetic, non-treated rats that were injected intraperitoneally (i.p.) once with citrate buffer (0.1 M, pH 4.5); vanadium-treated group (Van.): non-diabetic, vanadium-treated rats that were i.p. injected with citrate buffer as control group, and received vanadyl sulphate of 0.64 mmol/kg body weight, freshly dissolved in 1 ml of distilled water daily through a nasopharyngeal tube (Haidara et al., 2014); Diabetic type 1 group (D1): DM was induced in rats by a single i.p. injection of STZ in a dose of 65 mg/kg. Rats showed glucose level above 300 mg/dl were considered to be diabetics and included in the experiments (El Karib, 2014; Haidara et al., 2004); T1DM and insulin group (D1 + Ins.): rats were made type 1 diabetic as in T1DM group and received Mixtard insulin subcutaneously in a dose of 0.75 IU/100 g weight in 0.75 ml volume once daily (Haidara et al., 2015), after 48 h of diabetic induction; T1DM and vanadium group (D1 + Van.): T1DM rats received the same dose of vanadyl as in vanadium group after 48 h of induction of T1DM; T1DM and vanadium and insulin group (D1 + Van. + Ins.): T1DM rats received both vanadium and insulin with the same doses as in previous groups.

At the end of the 8th weeks of the experiment, 5 ml retro-orbital blood samples was obtained under anaesthesia using 40 mg kg⁻¹ sodium thiopentane, i.p., after fasting. Blood was collected into plain tubes, then allowed to clot for 20 min then centrifuged at 14,000 rpm for 10 min for serum separation. Then sera were stored at −80°C, for subsequent measurements of biochemical parameters. After withdrawal of the blood samples, the knee joints were opened, dissected, and fixed in glutaraldehyde and kept for electron microscopy examinations.

Measurement of IL-6, TNF-α cytokines

Rat interleukin-6 ELISA kit, IL-6 for serum, plasma and tissue culture supplements (Ray Biotech Inc., Mfr. No. ELR-IL6-001) and rat tumour necrosis factor alpha (TNF-α ELISA kit BIOTANG Inc., Cat. No. R6365 were used as recommended by the manufacturer.

Measurements of SOD and TBARS

Superoxide dismutase (SOD) assay kit, rat, Cayman Chemical, Cat. No. 706002 and thiobarbituric acid reactive substances (TBARS) assay kit, Cayman Chemical, Item Number 10009055 were used as recommended by the manufacturer.

Scanning electron microscopy

Different specimens were taken from articular cartilage of all treated rats and subjected for ultrastructural examinations with scanning electron microscope. Ultra-structural micrographs are representative of the changes seen in different treated groups. The specimens were fixed with 2.5% (wt/vol) sodium cacodylate-buffered glutaraldehyde, pH 7.2 at 4°C for 2 h. Specimens were also post fixed in 1% sodium cacodylate-buffered osmium tetroxide, pH 7.2 for 1 h. After washing and dehydration in ascending series of ethanol, critical-point drying was performed using the EMITECH-K850 critical-point drying unit. The specimens were mounted on aluminium stubs with double-sided tape and silver glue and then sputter coated with gold by BOC EDWARDS SCancoAT. The specimens were observed using a Jeol field emission scanning electron microscope JSM-6390LV.
Statistical analysis
Data are presented as mean ± SD. Comparison of data was analysed using Graph pad Prism software, version 5. Comparison between the groups was performed by one-way analysis of variance, followed by Tukey’s post-hoc test. Probability (p) values of <0.05 were considered to be significant.

Results
Changes in blood glucose level
Figure 1 shows a significant (p<0.0001) increase in blood glucose levels in diabetic group (371 ± 27 mg/dl) in comparison to the control group (92 ± 5 mg/dl). Compared to diabetic group, administration of vanadium and or insulin caused a significant (p<0.0001) decrease in glucose levels. In addition, insulin alone or with vanadium, but not vanadium alone, were able to lower blood glucose to levels comparable to the control group.

Osteoarthritis development in rats by induction of diabetes
Eight-week post-induction of diabetes, tissue preparations for histological examination under scanning electron microscopy (Figures 2 and 3) from the articular cartilage of the knee joint of the sacrificed T1DM rats (Figures 2C and 3C) revealed OA development compared to the non-diabetic healthy control rats (Figures 2A and 3A). The pathological changes revealed a massive destruction of the articular cartilage caused by T1DM such as atrophic chondrocytes inside distorted lacunae that are surrounded by damaged territorial matrix (Figure 2C), compared to normal structures in control tissues (Figure 2A). Furthermore, scanning electron micrographs of T1DM rats’ collagen fibers showed extensive damage and deterioration in fibrils forming sheets (Figure 3C), whereas normal fibrils forming sheets of collagen fibers were depicted in control rats (Figure 3A).

Insulin and vanadium protect the knee joint against T1DM-induced osteoarthritis
A substantial protection was obtained when insulin was given to diabetic rats (Figures 2D and 3D). 48 h after induction of diabetes, treating diabetic group with insulin for 8 weeks protected the OA ultrastructure of the articular cartilage to become much less damaged comparable to the control group. Scanning electron micrographs of insulin-treated tissues showed a normal territorial matrix and restoration of the leaving lacunae most of its normal size and regular shape, with a clear improvement in the healing of chondrocytes (Figure 2D). In addition, collagen fibers showed intact fibril sheets, but still with some focal damage (Figure 3D).

Administration of diabetic group with vanadium for 8 weeks differentially protected the ultrastructure of the articular cartilage (Figures 2E and 3E). Electron microscopy results revealed a better protection of leaving lacunae and territorial matrix than chondrocytes and collagen fibers (Figures 2E and 3E). However, focal damage of leaving lacunae and atrophic chondrocytes are still shown. Strikingly, co-administration of insulin and vanadium protects the articular cartilage of the diabetic rats where it showed normal chondrocytes, leaving lacunae, territorial matrix and collagen fibres (Figures 2F and 3F). The above treatments were compared to control groups, non-diabetic “vehicle-treated” (Figures 2A and 3A) and non-diabetic “vanadium-treated” rats (Figures 2B and 3B) that showed normal structures of the articular cartilage.

Insulin and vanadium inhibit T1DM-induced cytokines and oxidative stress
Proinflammatory cytokines are known to be involved in the pathology of diabetes (Senn et al., 2002; Shoelson et al., 2006; Southern et al., 1990) and OA (Doss et al., 2007, Stannus et al., 2010). To investigate whether vanadium and/or insulin can suppress the release of the proinflammatory cytokines in T1DM, we measured the blood level of TNF-α and IL-6, 8 weeks after T1DM induction. T1DM caused a 2-fold increase in TNF-α and 4-fold increase in IL-6 that were significantly inhibited by insulin or vanadium or vanadium plus insulin (Figure 4A and B).

Administration of insulin and vanadium significantly decreased IL-6 in comparison either to diabetic rats administer either insulin (p<0.0001) or vanadium (p<0.001) alone. Using post-hoc testing, least significant difference (LSD) for IL-6, it was found that there was a significance difference between diabetic group that received insulin and vanadium (p<0.0001) or diabetes and insulin (p<0.0001) or diabetes and vanadium (p<0.0001) alone. }

Insulin and vanadium administration to diabetic group significantly decreased TNF-α in comparison either to diabetic rats administer either insulin (p<0.0001) or vanadium (p<0.0001) alone. Using post-hoc testing (LSD) for
TNF-α, it was found that there was a significance difference between diabetic group that received insulin and vanadium in comparison to either diabetes and insulin ($p<0.0001$) or diabetes and vanadium ($p<0.0001$) alone.

To determine whether insulin and/or vanadium can modulate the oxidant and anti-oxidant biomarkers known to be affected by diabetes (Molinar-Toribio et al., 2014) and OA (Gutteridge, 1995), we measured the free radicals as TBARS, and SOD as biomarkers of oxidative stress by ELISA. Our results showed that diabetic induction augmented TBARS and suppressed SOD (Figure 5A and B). Insulin significantly augmented SOD and inhibited TBARS, whereas vanadium on its own was a weak stimulant to SOD and exerted no significant inhibitory effect on TBARS levels in T1DM group. Furthermore, co-administration of insulin with vanadium completely restored the levels of SOD and TBARS to the control levels (Figure 5A and B).

Administration of insulin and vanadium significantly decreased TBARS in comparison either to diabetic rats administer either insulin ($p<0.0001$) or vanadium ($p<0.0001$) alone. Using post-hoc testing (LSD) for TBARS, it was found that there was a significance difference between diabetic group that received insulin and vanadium in comparison to either diabetes and insulin ($p<0.0001$) or diabetes and vanadium ($p<0.0001$) alone.

Insulin and vanadium administration to diabetic group significantly increased SOD in comparison either to diabetic rats administer either insulin ($p<0.0001$) or vanadium ($p<0.0001$) alone. Using post-hoc testing (LSD) for SOD, it was found that there was a significance difference between diabetic group that received insulin and vanadium in comparison to either diabetes and insulin ($p<0.0001$) or diabetes and vanadium ($p<0.0001$) alone.

**Discussion**

The principal finding of our study was that insulin in combination with the insulinomimetic agent, vanadium inhibited OA induced by T1DM and suppressed the biomarkers of inflammation and oxidative stress known to be induced in diabetes and OA. Furthermore, insulin on its own was more potent than vanadium to protect against OA. To the best of our knowledge, these studies are the first to report a clear protective role for insulin and vanadium in OA induced...
Figure 3. Insulin and vanadium protected collagen fibres in the knee joint against OA in diabetic rats. Scanning electron micrographs (2000×) of rats’ collagen fibres in articular cartilage from the knee joint 8 weeks after the induction of diabetes by streptozotocin. A. Control, control vehicle injected rats. B. Van., vanadium-treated non-diabetic rats. C. D1, streptozotocin-injected rats (type 1 diabetic group). D. D1 + Ins., diabetic group treated with insulin. E. D1 + Van., diabetic group treated with vanadium. F. D1 + Van. + Ins., diabetic group treated with vanadium and insulin. Abbreviations: F, normal or intact collagen fibrils sheets; F1, damaged collagen fibrils sheets. Note that arrows point to focal damage of collagen fibril sheets.

Figure 4. Insulin and vanadium inhibit TNF-α and IL-6 in T1DM-treated rats. A. TNF-α and (B) IL-6 levels in blood from the groups (n = 6) were measured after 8-week treatment by ELISA. Control, control vehicle injected rats; Van., vanadium-treated non-diabetic rats; D1, streptozotocin-injected rats (type 1 diabetic group); D1 + Ins., diabetic group treated with insulin; D1 + Van., diabetic group treated with vanadium; D1 + Van. + Ins., diabetic group treated with vanadium and insulin. All results are the mean (±SD) of three experiments. Significance indicated: p < 0.05, P1: significant in comparison to control group, P2: significant in comparison to vanadium group, P3: significant in comparison to diabetic group.
Insulin and vanadium protect against OA

by diabetes. These conclusions were supported by the data indicating that STZ induced T1DM in rats caused a substantial destruction to the articular cartilage of the knee joint that was protected by insulin and vanadium.

Studies on experimental animals have demonstrated that administration of STZ, depending on its dose, extensively reduces beta cells mass and destroys pancreatic islet (Cam et al., 1997; Yanardag et al., 2005). Our results showed that vanadium and insulin administration causes decrease glucose level in diabetic group to control level which is in accordance with previous work that showed that regardless of the severity of diabetes, vanadium therapy needs a minimum level of plasma insulin, secreted endogenously, or received exogenously to assist vanadium to induce normoglycemia (Pirmoradi et al., 2014; Yanardag et al., 2005). In addition, the suppression of the growth body weight seen in the diabetic group was linked with the impaired carbohydrate metabolism as a source of energy (Pires et al., 2014, Wycherley et al., 2014). However, the suppressive action of vanadium on the appetite, via anorexigenic stimulation of the central nervous system and food restriction, was possibly the cause of reduced growth body weight in the vanadium-treated group (Marty et al., 2007). This is in agreement with our findings that showed no significant changes in the body weight of diabetic or vanadium-treated groups (data not shown).

We confirmed by electron microscopy examination the development of OA in the articular cartilage of the knee joint, which is known to be targeted by diabetes (El Karib, 2014; Musumeci et al., 2014; Niethard, 1986; Pottie et al., 2006). Tissue examination revealed a profound destruction of the cartilage structures such as chondrocytes, territorial matrix and collagen fibres.

Prolonged hyperglycemia and accumulation of glucose derived advanced glycation end products (AGEs) were proposed to contribute to OA development and insulin is reported to have anabolic effects on bone (Yan & Li, 2013). In addition, insulin was found to increase proteoglycan synthesis in primary chondrocytes tissue culture and increased proteoglycan and matrix synthesis in articular cartilage explants obtained from healthy pigs and diabetic human and mice (Cai et al., 2002). We therefore tested to see if insulin and its mimicking agent, vanadium is able to prevent articular cartilage injury in animals following diabetes. Indeed, our data showed that giving insulin or vanadium partially protected the knee joint injury. However, when both insulin and vanadium were given together, they protected the cartilage and the histological pictures were similar to the non-diabetic controls. Furthermore, It is important to note that the relative potencies for these agents in our T1DM-induced OA animal model were determined to be insulin + vanadium > insulin > vanadium. Our data also point to the importance of co-administering insulin and vanadium in preventing the increase of inflammatory and oxidative biomarkers levels above the control in diabetic-treated rats (Figures 4 and 5). It was also interesting to report that the relative potencies of vanadium were observed here to be well below that of insulin, which is in agreement with potent insulin over vanadium that we observed in the healing of the damaged cartilage.

Our protective approach, by co-administrating insulin and vanadium, that prevents the diabetic rats to develop OA concomitant with a complete inhibition of TNF-α, IL-6 and free radicals points to these cytokines and TBARS oxidative stress as very important proinflammatory biomarkers to monitor OA secondary to diabetes, including the healing progress of the disease. Indeed, our findings are in agreement with the previously published work on the role of TNF-α and IL-6 in the pathogenesis of inflammation induced bone loss, osteoclastic bone resorption and cartilage destruction (Fuller et al., 2002; Kapoot et al., 2011; Korczowska & Lacki, 2005) and the reports that proposed IL-6 and TNF-α as significant predictors of knee OA (Kou & Wu 2014; Livshits et al., 2009).

Taken together, our data support the conclusion that insulin protects against development of OA induced by T1DM and co-administration of vanadium with insulin is more effective to protect against the diabetic OA complications in STZ-induced OA in this rat model by lowering biomarkers of inflammation and levels of oxidative stress.
Declarations of interest

All authors declare that they have no conflict of interest.

This work was supported by grants of King Khalid University. KKU-Project No 2 (215).

References


