BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902019000318586

Article

Evaluating the effects of vanadyl sulfate on biomarkers of oxidative stress and inflammation in renal tissue of rats with diabetes type 2

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Vanadyl sulfate (VS) is an ingredient in some food supplements and experimental drugs. This study was designed to assay the effects of VS on biomarkers of oxidative stress and inflammation in renal tissue of rats with diabetes type 2. 30 male Wistar rats were divided into three equal groups as follow: non-diabetics, non-treated diabetics and VS-treated diabetics. Diabetes type 2 has been induced through high fat diet and fructose in the animals. Diabetic rats were treated with 25 mg/kgBW of VS in water for 12 weeks. At the end of study, glucose and insulin were measured using commercially available kits in serum and biomarkers of oxidative stress and inflammation in renal homogenates of animals were measured by related methods. Compared to controls, glucose and insulin were increased significantly in non-treated diabetic rats (*p-value <0.05*) that showed the induction of diabetes type 2 in rats. The results showed that in VS-treated diabetic rats compared to the non-treated diabetic group, vanadyl sulfate significantly reduced the glucose and insulin secretion and changed renal inflammatory and oxidative markers, except protein carbonyl so that we couldn't find any significant changes. Our study showed that vanadyl supplementation had positive effects on oxidative stress and inflammation biomarkers in kidney of diabetic rats.

Keywords: Vanadyl Sulfate, IL-1, IL-10. Oxidative Stress. Inflammations.

INTRODUCTION

Hyperglycemia can lead to diabetic problems and their socio-economic burden. Various tissues which have been exposed to high concentration of glucose may lead to changes in cell signaling, increment of oxidizing species or significant reduction in antioxidant capacity. Also, hyperglycemia has critical role in Advanced Glycation End products (AGEs) formation, as well as, secretion of the pro-inflammatory cytokines and cellular death (Volpe *et al*., 2018). Diabetic Kidney Disease (DKD) is one of the most common complications of diabetes, and its severity has increased throughout the world over the last decade due to the growing trend of diabetes, as it is becoming a major cause of death and mortality (Li, Ma, 2017; Liang, Cai, Chen, 2017). It has been documented that trace elements are required for proper function of metabolic pathways (Panchal, Wanyonyi, Brown, 2017; Mirhashemi, Aarabi, 2011). Vanadium, the 3d transition metal, has been considered as micro supplements for various biological functions in humans (Petanidis *et al*., 2013; Pessoa *et al*., 2015; Levina, Lay, 2017). Main attention to vanadium has focused on anti-diabetic effects but shifted to anti-cancer and anti-parasitic drugs (Levina, Lay, 2017; Kioseoglou *et al*., 2015). Vanadyl sulfate has been used in humans in the form of insulin-mimetic salt and thus controls both DM type 1 and type 2. Vanadyl sulfate protects

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from beta cells of Langerhans, and therefore provides insulin production and secretion that can improve blood glucose levels. (Dabros *et al*., 2004; Ahmadi *et al*., 2010; El-Shenawy, Refat, Fakihi, 2013) (1-3). Due to the rising occurrence of DKD, multiform study aimed at aborting and remedy is one of the universal priorities for researches. To the best of our facts, this is among the first studies to evaluate vanadyl sulfate on biomarkers of oxidative stress and inflammation in kidney of diabetic rats.

MATERIAL AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich Corporation.

Experimental animals

Healthy male Wistar rats, weighting about 180-210 g, were prepared from Experimental Animal Centre of Kashan University of Medical Sciences, and kept at standard conditions 22–24 ° C, 40–60% relative humidity and 12h light–dark cycle and fed with standard rat food and water ad libitum. The animals were protected in agreement with the ethics of care and use of experimental animals. The study protocol was permitted by the ethics committee of Kashan University of Medical Sciences (IR.KAUMS.REC.1395.161).

Induction of diabetes type 2 in rats

A typical diet of high fat (including 400 grams of sheep fat, 200 grams of sucrose, 18 egg yolks and 5 egg whites and 400 grams of rat chow, well combined and homogenized) and high fructose (including 25% fructose in drinking water) (HFFD: high fat and high fructose diet) was used for 12 weeks in order to generate diabetes type 2 model.

Studied groups design

30 rats were randomly divided into 3 equal groups as follows. The first group was normal rats that received standard rat chow and water without any HFFD/VS, and considered as control group. The 2nd group received HFFD without any treatment (non-treated diabetic group) and the 3rd group, received HFFD and 25 mg/kgBW vanadyl sulfate (diabetic treated with VS25).

Preparation of sera and kidney homogenate

After 12 weeks, at the end of their treatment periods, the animals were weighed and anesthetized using ether and killed by decapitation. Blood samples were collected; sera were separated from blood cells by centrifugation (Hettich D-78532, Tuttlingen, Germany) at 3000 rpm for 15 min, and stored at -80oC until the time of analysis. The kidneys were washed with cold saline (4 °C) and crushed into smaller pieces on ice, homogenized (10% W/V) with phosphate buffer with 0.1 molar and then centrifuged at 3000 g for 10 minutes at 4 °C. The supernatant solution was separated from the precipitate and used for measuring the parameters (Mirhashemi *et al*., 2009).

Glucose and insulin assay

Fasting blood glucose level, was determined in serum using commercially available kit (Pars Azmun, Tehran, Iran) by Mindray auto analyzer. Insulin level was quantified by ELISA method using kit of DiaMetra Company (Spello, Perugia, Italy).

Renal tissue oxidative biomarkers assay

Renal Protein Carbonyl (PC) and Malondialdehyde (MDA) were measured by colorimetric method using cayman chemical (USA) and Zellbio GmbH (Germany) commercially available kit with inter- and intra-assay CVs of lower than 5%, respectively. Superoxide Dismutase (SOD) activity was measured using Zellbio (Germany) company kit, too.

Inflammatory biomarkers assay

Renal tissue IL-1 and IL-10 concentrations were determined using ELISA kit (IBL International Company, Germany) with inter- and intra-assay CVs of 7.5 and 9.1%, respectively.

Statistical analysis

Data Analysis was performed using the statistical package for social science version 23 (SPSS Inc., Chicago, Illinois, USA). We conducted the Kolmogrov-Smirnov test to evaluate the normality of distribution of variables. Significance of differences was determined by ANOVA and posthoc Tukey's test. Results were presented as the

Mean ± SEM and statistically determined significant at $p \leq 0.05$.

RESULTS

Effect of vanadyl sulfate on glucose, insulin and HOMA-IR of studied groups

As it has been shown in Table I, there were statistically significant increase (p-value < 0.001) in glucose, insulin and HOMA-IR levels in 2nd group compared to controls (88.51%, 81.6% and 185.3% enhancements, respectively). Treatment with vanadyl sulfate resulted in a significant decrease in glucose, insulin and HOMA-IR levels in the 3rd group compared to the 2nd group by 49.21% (p-value < 0.001), 17.55% (p-value=0.012) and 31.9% (p-value = 0.002), respectively (Table I).

TABLE I - Effects of HFFD and vanadyl sulfate on fasting blood glucose, Insulin and HOMA-IR in experimental groups

Groups	Glucose (mg/dL)	Insulin(µIU/mL)	HOMA-IR
Control	110.91 ± 7.41	1.26 ± 0.04	0.34 ± 0.04
Diabetics	209.08 ± 11.60*	1.88 ± 0.11*	0.97 ± 0.07*
VS25	171.5 ± 9.46*	1.55 ± 0.07*	0.66 ± 0.05*

All groups were fed with HFFD except controls, for 12 weeks.
Treated groups received 25 mg/kg b.w. of vanadyl sulfate (VS25) in addition to HFFD for the same time
Data have been shown as Mean ±SEM, n=10.
*Indicated significant variations (p<0.05)

Effects of VS-treatment on oxidative stress biomarkers of renal tissue

According to table II and respect to control group, oxidative biomarkers including MDA and PC were statistically increased significant by 31.51% and 18.12% (p-value < 0.001) but SOD activity was decreased by 27.59% (p-value < 0.001) in the 2nd group. Vanadyl sulfate reduced MDA and PC in the 3rd (5.14% & 5.23%, respectively) group compared to the 2nd group, but these changes were statistically significant only for MDA (p-value = 0.029) not for PC (p-value = 0.28). Our results also showed that SOD activity was significantly increased by vanadyl sulfate in VS-treated group by 114.29% (p-value < 0.001).

TABLE II - Effects of HFFD and vanadyl sulfate on oxidative stress biomarkers in kidney of experimental groups

Groups	SOD (iU/mL)	MDA (µmol/L)	PC (nmol/mg)
Control	0.29 ± 0.01	6.82 ± 0.07	2.35 ± 0.09
Diabetics	0.21 ± 0.02*	8.94 ± 0.11*	2.87 ± 0.06*
VS25	0.45 ± 0.01*	8.48 ± 0.15*	2.72 ± 0.04

All groups were fed with HFFD except controls, for 12 weeks.
Treated groups received 25 mg/kg b.w. of vanadyl sulfate (VS25) in addition to HFFD for the same time.
Data have been shown as Mean ±SEM, n=10.
*Indicated significant variations (p<0.05)

Effects of treatment with vanadyl sulfate on renal inflammatory markers

Compared to control group, high fat and fructose diets led to an increase in IL-1 by 421.95% (p-value < 0.001) and a decrease in IL-10 by 41.3% (p-value < 0.001) in diabetic group. A significant (p-value < 0.001) reduction in IL-1 (21.18%) and increment in IL-10 (42.23%) were observed in VS-treated diabetic group compared to diabetics (Table III).

TABLE III - Effects of HFFD and vanadyl sulfate on inflammatory biomarkers in kidney of experimental groups

Groups	IL-1 (pg/mL)	IL-10 (pg/mL)
Control	**1.23 ± 0.07**	**3.51 ± 0.06**
Diabetics	**6.42 ± 0.08***	**2.06 ± 0.04***
VS25	**5.06 ± 0.07***	**2.93 ± 0.04***

All groups were fed with HFFD except controls, for 12 weeks.
Treated groups received 25 mg/kg b.w. of vanadyl sulfate (VS25) in addition to HFFD for the same time
Data have been shown as Mean±SEM, n=10.
*Indicated significant variations (p<0.05)

DISCUSSION

DKD has been considered as one of the most current complication of diabetes and clarified an important factor for death and mortality (Zac-Varghese, Winocour, 2018; Mirhashemi *et al*., 2016). It has been documented that after developing to renal failure, over 70% of DKD patients will die within five years (Zarqami *et al*., 2018), so new treatments for slowing down this progression are an urgent need that should be taken seriously. According to our knowledge, the present study was one of the first studies that evaluated the result of vanadyl sulfate on inflammatory and oxidative stress parameters in kidney of diabetes type 2 models. The first series of results showed that compared to controls, glucose and insulin were increased significantly in non-treated diabetic rats (p-value < 0.001) that showed the induction of diabetes type 2 in rats. Further, the results of vanadyl effects on these two items showed that vanadyl sulfate significantly reduced glucose and insulin secretion. In agreement with our study, Zarghami and colleges showed that vanadyl sulfate supplements may lead to glucose consumption improvement and feed efficiency (Zarqami *et al*., 2018). In kidney tissue of VS-treated rats, biomarkers of inflammations and oxidative stress were significantly improved, except for the amount of PC that did not change statistically. Oxidative stress and inflammation have been associated with the pathophysiology of diabetic nephropathy (Breyer, Kretzler, 2018; Sun *et al*., 2013). Studies about the experimental animal models of diabetes strongly implicated oxidant species as a major determinant in the pathophysiology of diabetic kidney disease (Breyer, Kretzler, 2018). In recent years, vanadium compounds have become important for a wide range of diseases and its interaction with cytokine interleukins has been shown (Lin *et al*., 2018; Sagoo, Gnudi, 2018). Using insulin signaling regulation, Liu and colleagues introduced a new complex of vanadium that resulted in improved performance of diabetic kidney rats. (Tsave *et al*., 2016). Other authors have also described new findings from various studies about the complex effects of insulin-mimetic vanadium in diabetic rodents (Pelletier *et al*., 2016; Liu *et al*., 2014; Jiang *et al*., 2016). Our study had some limitations. The main limitation of our study was lack of measuring the serum levels of vanadyl sulfate in animals due to financial constraints. Indeed, given the limited financial resource available for these projects, we did not evaluate some of the markers. Therefore, measurement of inflammatory cytokines and biomarkers of oxidative stress after intervention were reasonable in forthcoming studies. It may be concluded that vanadyl sulfate should be a necessary and beneficial molecule for the development of the therapeutic agents for the improvement of oxidative and inflammatory biomarkers in diabetic kidneys. Undoubtedly, further studies are needed to reveal the potential and/or possible toxicity of this compound in long-term use.

ACKNOWLEDGMENT

We respect the supports of Research Vice Chancellor of Kashan University of Medical Sciences for this project (NO#95158).

CONFLICT OF INTEREST

None confirmed.

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Received for publication on 05th August 2018
Accepted for publication on 30th September 2018