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A new oxovanadium complex enhances renal function by improving insulin signaling pathway in diabetic mice ☆

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ABSTRACT

Aim: Since vanadium complexes have insulin-mimetic effects and can be used to treat complications of diabetes, we aimed to screen a new oxovanadium complex with a low toxicity, and investigate its insulin-mimetic effects, as well as the mechanism of improvement to diabetic mouse renal function.
Methods: Cells were treated with oxovanadium complexes, and viability was assessed by MTT assay. Diabetic mouse model was established using alloxan. Blood urea nitrogen (BUN) and serum creatinine (SCr) in the mice were measured using an automatic biochemical analyzer, and blood glucose was measured using a Glucoval Compact meter. Expression of proteins related to the insulin signaling pathway in the renal cortex of mice was measured by Western blot analysis.
Results: Diabetic mice developed high blood glucose, BUN and SCr levels compared with control mice. The new oxovanadium complex with 3,5-dimethyl-pyrazolyl ligand, $VO(HB(3,5\text{-}Me_2pz)_3)(3,5\text{-}Me_2pz)(SCN)(SCNH)_2$, showed low toxicity and significantly reduced blood glucose, BUN and SCr levels in the diabetic mice. Additionally, p42/p44MAPK and Akt phosphorylation was markedly increased in diabetic mice and was decreased by treatment with the new oxovanadium complex. Caveolin-1 (Cav-1) expression was greatly decreased in diabetic mice and significantly increased after treatment with the new oxovanadium complex.
Conclusions: The new oxovanadium complex, with 3,5-dimethyl-pyrazolyl ligand, improves kidney function in diabetic mice, and its mechanism may involve regulation of the insulin signaling pathway.

1. Introduction

Diabetes is a common metabolic disease with patient numbers increasing each year. The clinical manifestations of diabetes include hyperglycemia, insulin resistance and complications such as hypertension, neuropathy, proliferative retinopathy and diabetic nephropathy (DN) (Alva, Gray, Mihaylova, & Clarke, 2013). DN is a type of microvascular complication which seriously affects the life quality of patients with diabetes. Multiple factors induce DN, including metabolic factors, hemodynamic factors, oxidative stress and genetic factors. While the pathogenesis of DN is still inconclusive (Xiao et al., 2013), dysregulation of the insulin signaling pathway may be involved.

Insulin plays key roles in the proliferation and differentiation of cells and the control of glucose homeostasis (Pirola, Johnston, & Obberghen, 2004). It mediates glucose uptake (Song et al., 2012), glycogen synthesis (Song et al., 2013), and lipid synthesis (Ito et al., 2013) in muscle, adipose, and hepatic tissues. Insulin plays a role through a specific insulin receptor (IR), which belongs to the family of tyrosine kinase receptors (Rains & Jain, 2011). The insulin molecule binds to the IR causing tyrosine autophosphorylation of the receptor. The activated IR directly phosphorylates insulin receptor substrates (IRS) on multiple tyrosine residues. Tyrosine-phosphorylated IRS proteins bind to phosphatidylinositol 3-kinase (PI3K), which is the main signal mediator of the metabolic and mitogenic actions of insulin, then activate the serine kinase PKB/Akt (Bloch-Damti & Bashan, 2005). These results in several of insulin's responses: GLUT4

☆ Conflict of interest: None.

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translocation to the membrane, glycogen synthesis via phosphorylation of GSK-3, and lipogenesis by up-regulating synthesis of the fatty acid synthase gene. Mitogen-activated protein kinase (MAPK) can also be activated by insulin. Phosphorylated IRS-1/2 recruits SOS and GRB-2, forms a complex with SHP-2, then activates membrane-bound Ras. Activated Ras leads to a kinase cascade, finally translocating ERK to the nucleus for gene expression (Saltiel & Kahn, 2001).

Caveolae are flask-shaped invaginations located in the plasma membrane and are rich in multiple signaling molecules. Caveolae play important physiological roles in cell metabolism, and participate in vesicular transport, signal transduction, and protein anchorage (Klein et al., 2009; Volonte & Galbiati, 2009; Felicetti et al., 2009). Caveolin-1 (Cav-1), a marker protein of caveolae, interacts with various signaling molecules via its scaffolding domain to regulate many signaling pathways. The IR is located at the same microdomain as Cav-1 in adipocytes and hepatic cells (Yamamoto et al., 1998), implicating physical and/or functional interaction between Cav-1 and the IR. Insulin signaling also depends on the integrity of caveolae. Destruction of caveolae disrupts insulin-stimulated phosphorylation of insulin receptor substrate-1 (Parpal, Karlsson, Thorn, & Stralfors, 2001).

Vanadium complexes are associated with maintaining normal body functions and the pathogenesis of some human diseases. It has been reported that vanadium has insulin-mimetic effects both *in vitro* and *in vivo* and improves hyperglycaemia. They have also been used in the treatment of diabetic complications such as obesity, hypertension and DN (Srivastava & Mehdi, 2005; Xie et al., 2009; Saha, Yoshikawa, & Sakurai, 2007; Adachi et al., 2006; Morsy, Abdel-Razek, & Osman, 2011). However, potential short- and long-term vanadium toxicity significantly slowed the application of its therapeutic use (Soveid, Dehghani, & Omrani, 2013; Shukla, Barve, Padhye, & Bhonde, 2006; Pillai, Subramanian, & Kandaswamy, 2013). High doses of vanadium are required for insulin-mimetic effects but have higher cytotoxicity, whereas low doses are relatively safe but lack anti-diabetic activity. The common side effects of vanadium complexes are dehydration, decreased fluid and food intake, diarrhea and loss of body weight. Therefore, current studies focus on synthesizing new oxovanadium complexes with lower toxicity and investigating the potential mechanism of anti-diabetic activity. It has been shown that many vanadium complexes activate several key components of the insulin signaling pathway, such as Akt/PKB and MAPK (Vardatsikos, Mehdi, & Srivastava, 2009; Mehdi, Vardatsikos, Pandey, & Srivastava, 2006; Cortizo, Molinuevo, Barrio, & Bruzzone, 2006; Chien, Mak, & Huang, 2006).

In this report, we show that $VO(HB(3,5\text{-}Me_2pz)_3)(3,5\text{-}Me_2pz)(SCN)(SCNH)_2$ (Xing et al., 2006), the new oxovanadium complex with 3,5-dimethyl-pyrazolyl ligand, has effective anti-diabetic activity with lower toxicity. This oxovanadium complex markedly reduced blood glucose, BUN and SCr levels in alloxan-induced diabetic mice. It reduced the phosphorylation of p42/p44 MAPK and Akt/PKB, and increased the expression of Cav-1 in the renal cortex of diabetic mice.

2. Materials and methods

2.1. Animals

Male Kunming mice (Grade: SPF), weighing 18 ~ 22 g, were purchased from Dalian Medical University (Dalian, China). They were housed ten/cage at 20 ~ 25 °C (12 h light/dark cycle), fed with a commercial pellet diet *ad libitum* and given free access to water according to the guidelines of the International Council for Laboratory Animal Science (ICLAS). Mice were starved for 24 h before inducing a diabetes model. Mice were sacrificed by cervical vertebra dislocation. The renal cortex tissue was isolated immediately and stored at −80 °C until processed for further processing. Only the number of mice necessary was used and tried our best to minimize suffering.

2.2. Reagents

Oxovanadium complexes were synthesized by the method described in previous studies (Xing, Sun, Yuan, et al., 2006; Xing, Aoki, Sun, Ge, & Niu, 2007; Xing et al., 2006). Oxovanadium (IV) complexes $VO(HB(pz)_3)(pz)(SCN)$ and $VO(HB(3,5\text{-}Me_2pz)_3)(3,5\text{-}Me_2pz)(SCN)(SCNH)_2$ with tris(pyrazolyl)hydroborate ligands were prepared by the reaction of $VOSO_4 \cdot nH_2O$, KSCN with $Na(HB(pz)_3)$, pyrazole, and $Na(HB(3,5\text{-}Me_2pz)_3)$ and methyl-substituted pyrazole in the solution of MeOH, respectively. Oxovanadium (IV) complexes $HB(pz)_3VO(acac)$ and $HB(3,5\text{-}Me_2pz)_3VO(acac)$ were prepared by the reaction of $VO(acac)_2$ (acac = acetylacetonato) with $NaHB(pz)_3$ (pz = pyrazole) and $NaHB(3,5\text{-}Me_2pz)_3$ in MeOH, respectively. The complexes above were characterized by IR, UV–vis, NMR, elemental analysis and X-ray diffraction (Fig. 1). In addition, $VO(acac)_2$ was used as a reference compound.

Alloxan was obtained from Biosharp. Rabbit polyclonal anti-caveolin-1 (N-22), horseradish peroxidase-labeled goat anti-mouse and anti-rabbit secondary antibodies were obtained from Santa Cruz Biotechnology. Mouse monoclonal anti-β-Actin was obtained from Boster Biotechnology. Rabbit monoclonal anti-Akt, rabbit polyclonal anti-phospho-Akt, Rabbit monoclonal anti-p44/42 MAPK and mouse polyclonal anti-phospho-p44/42 MAPK, were obtained from Sigma. Other biochemical reagents were from Promega.

2.3. Cell culture

Mouse fibroblast cell line 3T3-L1 and rat hepatoma cell line CBRH-7919 were obtained from SIBCB and cultured in Dulbecco's modified Eagle's medium (DMEM/F12, Invitrogen), containing 10% fetal bovine serum (Hyclone, Logan, UT), 25 mM glucose, 100 μg/ml penicillin and 100 μg/ml streptomycin at 37 ºC in the presence of 5% CO_2.

2.4. Cell viability assay

Cell viability was measured using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Blue formazan was only active in live cells, which was metabolized from colorless MTT by mitochondrial dehydrogenases.

Cells were seeded in 96 multi-well plates (5×10^3 cells/well). When cells reached 70% confluence, the medium was replaced with new DMEM/F12 with oxovanadium complexes at different doses (1, 10, 100 and 1000 μM) at different time points (12, 24, 36 and 48 h). In this *in vitro* MTT assay, 1.3, 13, and 130 μg/L elemental vanadium content in $VO(acac)(HB(pz)_3)$ and $VO(HB(pz)_3)(pz)(SCN))$, 1, 10, and 100 μg/L elemental vanadium content in $VO(acac)(HB(3,5\text{-}Me_2pz)_3) \times CH_3CN$, 0.8, 8, and 80 μg/L elemental vanadium content in $VO(HB(3,5\text{-}Me_2pz_2)_3)(SCN)(SCNH)_2$ were added to cells. MTT assay was carried out according to the manufacturer's protocol (Key Gen, Nanjing, China). MTT (5 mg/ml) was added to the plates and incubated at 37 °C in a 5% CO_2 atmosphere for 4 h. The reaction was stopped by the addition of DMSO. Then the extraction was measured at 490 nm with multi-well reader (Labsystems Multiskan Ascent), and the values were normalized and plotted as percentage of control cells. The percentage viability was calculated.

2.5. Establishment of diabetic mouse model and oxovanadium complex administration

An experimental diabetic mouse model was induced in 4 week old male Kunming mice using alloxan monohydrate. After a 24 hour fast, diabetes was induced in mice via alloxan (200 mg/kg, i.p.) injection for four consecutive days. Control group mice were injected with the same volume as the diabetes group using 0.9% saline buffer. Two days after alloxan injection, blood samples were collected through the tail vein, and blood glucose level was

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