ORIGINAL ARTICLE

Effects of oral vanadium on glycaemic and lipid profile in rats

Syed Zubair Hussain Shah,[1] Abdul Khaliq Naveed,[2] Amir Rashid[3]

Abstract

Objective: Vanadyl sulphate, an inorganic tetravalent salt of transition metal vanadium is conventionally used to treat diabetes and by athletes as body-building supplement. Vanadyl sulphate is a constituent of many supplements and herbal preparations available over the counter in many parts of the world. In this study the efficacy of the salt as hypoglycaemic agent and its effects on lipid profile were determined when administered in therapeutic dose range (in humans) to healthy Sprague Dawley rats for a considerable duration.

Methods: One hundred and five rats were randomly divided into three groups of 35 rats each. Animals of all three groups were provided normal rodent diet and water ad libitum. Group I animals were administered 0.5 ml plain water through oral gavage while group II and group III rats, 0.25mg/Kg/day and 1.2mg/Kg/day vanadyl sulphate respectively for 24 weeks. At the end of 24 weeks intra-cardiac blood sampling was done and blood glucose, insulin and lipid profile were measured.

Results: There was significant decrease in plasma glucose, insulin and HDL-c levels while LDL-c, TGs and TC levels were significantly increased in a dose dependent manner in treated groups.

Conclusion: Our study showed that vanadyl sulphate possesses hypoglycaemic effect in healthy rats while insulin levels are also decreased which may be secondary to hypoglycaemia. Moreover it causes unfavorable derangement of lipid parameters in treated rats. In conclusion vanadyl sulphate though contains significant hypoglycaemic effects; its use in humans may be re-evaluated to establish its safety in relation to lipid profile.

Keywords: Vanadyl sulphate, Hypoglycemia, Dyslipidemia, Sprague-Dawley rats. (JPMA 66: 1592; 2016)

Introduction

Vanadyl sulphate (VOSO4), an inorganic salt of tetravalent vanadium (V+4), being used as anti-diabetic agent, is thought to have glucose lowering effects, through its insulin mimetic properties,[1] in animal and human subjects.[2,3] Salt is also used as nutritional supplement for development of muscles by athletes.[4] Vanadyl sulphate is constituent of many supplements and herbal preparations available over the counter in many parts of the world. Vanadium is thought to be a cofactor in many enzymatic reactions.[4] The mechanism of action can also be attributed to its interaction with enzymes like phosphatases and kinases, and alteration in concentration of reactive oxygen species (ROS) interfering with phosphatases.[5] Some studies show that vanadium exhibit insulin like effects in humans by decreasing gluconeogenesis and increasing deposition of glycogen.[4] According to Thomas Scior and others vanadium can be bioequivalent and replace phosphate in metabolic pathways.[6] Vanadium is found and acts in vivo in form of a vanadyl cation.[4] Many of such effects lead to a favorable outcome regarding plasma glucose levels especially in diabetes mellitus and dyslipidaemias.[4] The insulin mimetic effect is thought to be due to vanadium's inhibitory effect on protein tyrosine kinase but generally the mechanism of vanadium action remains obscure.[4] Vanadyl sulphate has been implicated in treatment of type 2 Diabetes Mellitus (DM) and is reportedly less toxic than (V2) vandate.[4] VOSO4 is being used in humans as insulin-mimetic salt and thus controls both type 1 and type 2 DM.[7] VOSO4 protects the beta cells[8] of Islets of Langerhans thus promoting insulin synthesis and release resulting in improved glycaemic control.[8] Vanadium exerts its affects in vanadyl (V+4) form and plays role primarily in glucose uptake in peripheral tissue.[9] Recent studies show that vanadium is quite efficacious against hyperglycaemia.[10] Vanadium increases the expression and multimerization of adiponectin in rat adipocytes by up regulating peroxisome proliferator-activated receptor (PPARγ).[11,12] Vanadium complexes increase levels of PPARγ primarily by suppressing its degradation. Moreover protein-protein interaction between PPARγ and vanadium binding chaperone is induced leading to supportive effect on adiponectin levels increase.[11] Adiponectin increases glucose uptake by cells and thus lowers plasma glucose levels. Vanadium increases the release of leptin

[1,3]Department of Biochemistry & Molecular Biology, Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi, [2]Department of Biochemistry, Islamic International Medical College, Riphah International University, Rawalpindi, Pakistan.

Correspondence: Syed Zubair Hussain Shah.
Email: zubair@amcollege.nust.edu.pk

because it is shown that the golgi complexes remain normal ring shaped and increase in number in diabetic rats treated with vanadium[13] as compared to non-treated diabetic group. Leptin acts through central nervous system and lowers plasma glucose levels through increase glucose uptake by muscle and brown adipose tissue and decreased production by liver. It is also observed that vanadium possibly interacts with surface proteins on cell membranes and partly the lipids on cell surface to bring about its action.

On the other hand some studies found that vanadium is not effective against hyperglycaemic states to the extent shown by some other researchers. Another recent study shows that vanadium is not effective against Diabetes mellitus at all.[14]

Vanadium salts are believed to exert pancreatic beta cells sparing or protective effect by preventing these cells from hyperglycaemic oxidative stress which is also evident in histological and ultra-structural studies of beta cells.[15] Beta cell loss is also attributed to oxidative damage to endoplasmic reticulum caused by increased circulating free fatty acids in type 2 DM. Free fatty acids liberated in increased amounts due to resistance to insulin or insulin deficiency, cause increase oxidative stress to Endoplasmic Reticulum (ER) which cause some proteins to go unfolded. Unfolded proteins then trigger a pathway leading to down regulation of pro-survival chaperone immunoglobulin heavy chain binding protein, 78kDa glucose regulated protein and up regulation of expression of apoptotic proteins leading to more beta cells apoptotic deaths and less insulin synthesis and secretion. While vanadium not only prevents beta cells from destruction by hampering this fatty acid mediated pathway but also ameliorates Ca+2 homeostasis. Thus increasing insulin release and better glycaemic control.[16,17] Ultra structure beta cell studies show lymphocyte infiltration, cytoplasmic vacuolization and nuclear picnosis in diabetic rats while these changes were found absent in vanadyl sulphate treated diabetic rats indicating towards its beta cell protective role.[8] Thus, the favourable glycaemic effects of vanadium can partially be attributed to increased insulin release by protective effect over the beta cells of pancreatic islets.[18] So serum insulin levels are expected to be raised as compared to the controls in our study, though other studies show it is insulin sensitization which is increased by vanadium and not the insulin secretion.[19]

In DM either type 1 or 2, vanadium compensates decreased insulin activity by activation of insulin signaling pathways, however this study tried to explore that whether it alters quantum of insulin action in healthy animals, its effects on insulin and plasma lipid levels.

We looked for effects of vanadium in non-diabetic animals and tried to explore whether it possesses hypoglycaemic effects in healthy animals. It has been observed that the healthy rats are more susceptible to effects of vanadium as compared to the diabetic rats.[14] As vanadium inorganic compounds are found in earth crust and significant quantities may contaminate drinking water in certain areas, its effects on healthy animals would be important to consider.

Materials and Methods

This was a randomized control trials panning one year and started in November 2012. The sampling of animals was non probability convenience sampling and included animals of six to eight weeks age and 230±20g weight. Animals were kept under standard conditions with a daily photo period of 12 hours light and 12 hours dark at temperature 23±5°C at animal house at Quaid e Azam University (QAU). One hundred and five rats were randomly divided into three groups of 35 rats each. Animals of all three groups were provided normal rodent diet and water ad libitum. Group I animals were administered 0.5 ml plain water through oral gavage[20] while group II and group III rats,0.25mg/Kg/day and 1.2mg/Kg/day vanadyl sulphate respectively for 24 weeks. Average weight of a rat was calculated for each group and dose was calculated for single rat. Then one liter aqueous solution of salt was made containing per-day dose for single rat per 0.5ml of water. Thus two solutions of different concentration i.e. 125mg and 600mg per liter of aqueous solution were prepared for group II and III respectively and administered to rats as single daily dose regularly for 24 weeks.

At the end of the treatment period terminal intra cardiac blood sampling was done. Total 3.5 ml blood was drawn out of which almost 1.5 ml was taken in Ethylene-diamine-tetra-acetic acid (EDTA) and sodium fluoride bottles. Bottles were rotated gently and centrifuged at 3000rpm for 5 minutes. Plasma was taken and transferred to another bottle for glucose analysis by Selectra E auto analyzer. Approximately 2ml of blood was transferred to the tubes containing serum gel and clot activator, centrifuged for 10 minutes at 3000 rpm and serum obtained was used for measuring lipid profile and Insulin levels. Plasma glucose levels were measured by glucose oxidase method[21,22] using a commercial ready to use kit by Globe Diagnostics S.rl, Milan, Italy on Vitalab Selectra E. Rat Insulin Enzyme Immunoassay Kit (EIA) manufactured by SPI BIO France was used for estimation of insulin

concentration in samples.

The serum was used for enzymatic colorimetric estimation of all parameters of lipid profile including triglycerides (TGs), Total Cholesterol (TC) and high density lipoproteins-cholesterol content (HDL-c) except low density lipoproteins- cholesterol content(LDL-c) and very low density lipoproteins- cholesterol content (VLDL-c) which were calculated using Friedewald formula. The statistical analysis was done by SPSS 15 and significance of differences was determined by ANOVA and posthoc Tukey's test.

Results

The comparison of mean glucose and insulin levels among three groups at the end of 24 weeks is shown in Table. Both glucose and insulin levels gradually decreased with increasing dose of vanadyl sulphate within usual therapeutic dose range. A significant decrease in plasma glucose levels in animals of group II as compared to controls and among those of group 3 as compared to those of group II clearly demonstrate the hypoglycaemic effect of Vanadium (+4) even in non-diabetic animals. A significant dose dependent decrease in serum insulin levels was observed in treated groups contrary to our hypothesis.

The total cholesterol (TC), triglycerides (TGs), low density lipoproteins- cholesterol content (LDL-c), very low density lipoproteins- cholesterol content (VLDL-c) were all raised in a dose dependent manner in treated groups. While the levels of high density lipoprotein- cholesterol content (HDL-c) was decreased in treated groups. As the dose increased all parameters of lipid profile except (HDL-c) increased gradually from group II to group III while the (HDL-c) declined gradually. In our study vanadyl sulphate showed hypoglycaemic effect in healthy rats when administered for longer durations in a low dose range. The salt also decreased circulating insulin levels in a dose dependent fashion probably secondary to hypoglycaemia and showed undesirable effects on plasma lipid parameters.

Discussion

Vanadyl sulphate showed a lowering effect on plasma glucose levels in normal healthy Sprague Dawley rats in this study. This is evident that vanadium (IV) reduces plasma glucose levels and a number of pathways have been suggested for this effect to take place, like activation of PPARγ,[11,12] expression of adiponectin,[11,12] sparing effect on beta cells,[8] increase in release of leptin[13] and activation/ phosphorylation of protein kinase B (PKB) pathway.[23] There have been two views on the effects of vanadium on glucose metabolism. Some advocate that vanadium causes decrease in plasma glucose levels until euglycaemia is achieved in hyperglycaemic subjects[24] while others suggest that it causes even hypoglycaemia and lowers plasma glucose beyond euglycaemia.[25] Most of the studies have been carried out on diabetic subjects and fewer or negligible number of studies addressed normal healthy subjects. Present study was planned on normal (non-diabetic) Sprague Dawley rats and to observe the behavior of vanadium in euglycaemic state. From this study it is deducted that in a dose range equivalent to 20-100mg vanadyl sulphate per day in humans, vanadium causes dose dependent hypoglycaemia in euglycaemic rats. The normal glucose levels were taken as average plasma glucose levels in control group i.e. (196±25 mg/dL) and a significant decrease was observed in rats of group II and a further significant decrease was observed in group III animals. This supports the belief that vanadium also lowers plasma glucose levels in absence of insulin resistance. This suggests that mechanisms other than that of sparing beta cells are significantly effective to alter glucose metabolism. Insulin levels also decreased significantly in a dose dependent manner apparently suggesting decreased requirement or decreased stimulation of insulin release in presence of vanadium (IV) in circulation. Vanadium has also been proposed to exert sparing effect on beta cells15 in type 1 and type 2 diabetic subjects (attributed to oxidative stress to endoplasmic reticulum

Table: Results showing glycaemic and lipid profile of group I, II and III rats.

	Mean ± SD Values of all Groups			
Variables	**Group I (n = 32)**	**Group II (n = 31)**	**Group III (n = 30)**	**P value**
Plasma Glucose (mg/dL)	196 ± 25	167 ± 21	133 ± 20	< 0.05
Serum Insulin(µg/L)	0.039 ± 0.005	0.036 ± 0.002	0.033 ± 0.002	< 0.05
TG (mg/dL)	88.5 ± 3.5	113.3 ± 1.2	150.4 ± 8.8	< 0.05
TC (mg/dL)	41 ± 1.5	53 ± 4.6	68.8 ± 3.9	< 0.05
LDL-c (mg/dL)	8.5 ± 1.1	17 ± 1.9	27.4 ± 2.3	< 0.05
VLDL-c (mg/dL)	7.7 ± 0.39	10.0 ± 1.2	13.1 ± 0.8	< 0.05
HDL-c (mg/dL)	14.7 ± 1.2	12.8 ± 1.5	10.8 ± 1.5	< 0.05

TG: Triglycerides. TC: Total cholesterol. LDL-c: Low-density lipoprotein cholesterol content. VLDL-c: Very low-density lipoprotein cholesterol content. HDL-c: High-density lipoprotein cholesterol content. SD: Standard deviation.

caused by increased circulating free fatty acids in type 2 DM[16,17]). Some believe that insulin secretion is increased by protective effect of vanadium on pancreatic islet cells[18] while others suggest that it only increases insulin sensitivity and not the secretion.[19] In present study insulin levels were expected to increase with increasing dose of vanadyl sulphate but the results were contrary to the expected outcome. Possible reason to this may be that the subjects were normal (non-diabetic) and thus there was no damage to beta cells which was to be reversed and thus no increase in insulin levels were seen. Second possibility is that the insulin mimetic effects reduce plasma glucose levels and decrease stimulation for insulin secretion resulting is dose dependent gradual decrease in serum insulin levels. It can be deducted from results of our study that in non-diabetic subjects with un-altered carbohydrate metabolism vanadium tends to reduce insulin secretion. It is proposed that this effect may be secondary to decrease in requirement of insulin which is in part being fulfilled by insulin mimetic effects of vanadium.

Studies have shown that vanadium treatment increases circulating triglycerides (TGs)[26] and increases other lipid parameters.[27,28] It has been observed that vanadium deranges cellular lipid metabolism and enhances pathological lipid accumulation in epithelial cells of intestine.[28] In another study vanadium failed to ameliorate dyslipidaemia in diabetic rats.[29] The results of our study are consistent with the findings of these studies.

On the other hand long term oral vanadium administration to diabetic subjects has recently been shown to have lowering effects on lipid parameters other than HDL-c levels which are shown to increase with un-altered TG levels.[30] Similar type of lipid lowering effects have also been demonstrated recently in occupationally exposed individuals to vanadium.[31] There are a number of other studies which advocate benefit of vanadium supplementation for correction of lipid disorders.[32,33] except few others which advocate no benefit at all.[29] Protein Kinase B activation pathway has been suggested to be responsible for anti-lipolytic effect of vanadium through decrease in phosphorylation of hormone sensitive lipase in adipose tissue.[34] Moreover up-regulation of fatty acid translocase in liver by vanadium has been shown to reduce TG accumulation in liver of diabetic rats.[35] Results of present study are contrary to findings of these studies and possible reason may be an altered role of vanadium in normal healthy metabolic state. Most of above mentioned studies have been carried out on diabetic subjects in whom lipid metabolism is already altered. Irrespective of the fact that whether vanadium has got a beneficial or otherwise role in management of lipid disorders in diabetes mellitus, in this study the effects of vanadium are observed on lipid profile in normal un altered metabolic state in normal healthy rats. The effort was to establish a positive correlation between vanadium supplementation and lipid profile benefits so that the element could be suggested for further trials as an independent lipid lowering salt. But the results showed that it rather deranges lipid parameters and may be unsuitable for consideration as anti lipidaemic agent. In the nutshell despite its considerable hypoglycaemic effects, use of vanadyl sulphate in humans needs to be studied further to ascertain its safety regarding lipid parameters.

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