Comparative Effects of Glimepiride, Vanadyl Sulfate and Their Combination on Hypoglycemic Parameters and Oxidative Stress

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Abstract: The present study was performed to evaluate the effect of glimepiride, Vanadyl Sulfate (VOSO₄) or their combination on glycemic status and oxidative stress in STZ-diabetic rats and to investigate the possible mechanism of action of these drugs. Another aim of this study is to determine whether there is a possible interaction between certain chosen trace element (VOSO₄) and glimepiride as representative to oral hypoglycemic agents. Treatment of STZ-diabetic animals with glimepiride (10 mg/kg, p.o.) or VOSO₄ (15 mg/kg, i.p.) decreased serum glucose level and increased liver glycogen content. Glimepiride increased serum insulin level and glucose (6 mmol/L)-stimulated insulin secretion from isolated rat pancreatic islets. Vanadyl sulfate did not affect serum insulin level or glucose (6 mmol/L)-stimulated insulin secretion from isolated rat pancreatic islets. Glimepiride and VOSO₄ increased plasma GSH level, plasma SOD level and decreased serum TBARS level. Combination of VOSO₄ with glimepiride did not improve the effect of glimepiride alone on any of the measured parameters when given in the selected doses indicating no significant interaction between these two drugs on the selected parameters. It could be concluded that concurrent administration of VOSO₄ with glimepiride does not produce any additive effect on any of the measured parameters. Therefore, VOSO₄ can be taken safely as a micronutrient in diabetic patient treated with glimepiride without fear of any serious reactions.

Keywords: Diabetes, glimepiride, hypoglycemic parameters, oxidative stress, vanadyl sulfate

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized mainly by hyperglycemia (Barnett, 2009) which results from either a decrease in insulin secretion or from insulin resistance in the target tissues (Tian et al., 2006).

Diabetes Mellitus is the major cause of many serious complications such as cardiovascular disorders (Ewais et al., 2005), diabetic foot (Demiot et al., 2006), peripheral neuropathy (Boulton et al., 2005), nephropathy (Yin et al., 2004), retinopathy (Santoso, 2006) and depression leading to mortality (Antai-Otong, 2007; De Groot et al., 2007).

In addition, oxidative stress has been reported to play an important role in T2DM (Siddiqui et al., 2005; Shukla et al., 2007).

Administration of oral hypoglycemic agents is necessary for achievement of the required glycemic control (DeFronzo, 1999). Glimepiride is an important oral hypoglycemic agent of the sulfonylurea group (See et al., 2003). However, glimepiride suffers from severe side effects such as gastrointestinal disorders (Paice et al., 1985), hypersensitivity reactions (Paice et al., 1985) and severe hypoglycemia which may be life-threatening (Ferner and Neil, 1988).

Therefore, there is a need to search for newer and alternative therapy for T2DM to be more effective and less toxic.

Vanadium is a trace element present in low concentration in animals (Zorzano et al., 2009) and mammals (Bolkent et al., 2005), which has been previously reported to exert antidiabetic action (Cohen et al., 1995; Goldfine et al., 1995; Boden et al., 1996; Siddiqui et al., 2005).

Consequently, it deemed necessary in the present study to examine the possible antidiabetic and antioxidant effects of vanadyl sulfate as well as glimepiride alone and in combination. The study of co-administration of vanadyl sulfate with glimepiride seemed of importance.
MATERIAL AND METHODS

Animals: Adult Sprague Dawley male rats weighing 150±20 g for in vivo experiments and 200±20 g for in vitro experiment were used in this study. Animals were obtained from National Research Center, Cairo, Egypt. Rats were housed in plastic cages and were maintained under conventional laboratory conditions throughout the study. They were fed standard pellet chow (El-Nasr chemical Co., Abu Zaabal, Cairo and Egypt) and allowed water ad libitum. All procedures in this study were carried out according to guidelines of Ethics Committee of Faculty of Pharmacy, Cairo University.

Drugs and chemicals: STZ was purchased from Sigma-Aldrich Chemical Co. (U.S.A.). Glimepiride was provided as a gift from SEDICO Company, Egypt. It was suspended in 2% Tween 80 and orally administered in a dose of 10 mg /kg (Ladriere et al., 1997). For in vitro experiments a dose of 10 μmol/L was chosen according to (Hu et al., 2001). Vanadyl sulfate was purchased from Sigma-Aldrich Chemical Co. (U.S.A.).

Induction of experimental diabetes: Experimental diabetes was induced in 18 h fasted rats by single i.p. injection of Streptozotocin (STZ) in a dose of 50 mg/kg (Hounsom et al., 1998) freshly prepared in cold 0.1 M citrate buffer (pH 4.5). STZ-injected rats were provided with 5% glucose drinking solution for the first 24 h to ensure survival (Hajduch et al., 1998). Normal control group was injected with citrate buffer alone. Animals were considered diabetic when their blood glucose level exceeded 250 mg/dL (Cam et al., 2003) and were included in the study after 72 h of STZ injection.

Isolation and incubation of rat pancreatic islets: Pancreatic islets were isolated following collagens digestion technique according to the method of (Lacy and Kostianovsky, 1967). The islets were pre-incubated into Wassermann tube containing fresh Krebs-Ringer-HEPES (KRH) solution and incubated at 37°C for 30 min in a shaking water bath for adaptation (LACY et al., 1968). Finally, Batches of 5 islets were picked up and incubated in small tubes, each containing 1 mL KRH buffer supplemented with 0.5% bovine serum albumin, glucose 6 mmol/L and the test drug.

Experimental design:

In vivo experiment: Rats were divided into 5 groups, each consisting of 6-8 rats. Four groups of them were made diabetic by STZ (50 mg/kg i.p.), the remaining group was considered as normal control and received an equivalent volume of citrate buffer. These diabetic rats were randomly classified into 4 groups; one of them received 1% Tween 80 and was considered as diabetic control. The remaining 3 groups received glimepiride (10 mg/kg p.o.), vanadyl sulfate (15 mg/kg i.p.) or combination of both glimepiride and vanadyl sulfate, respectively. These treatments were started 72 h after STZ administration daily for 2 weeks.

In vitro experiment: Isolated rat pancreatic islets were divided into 4 groups. Each group consists of 5-6 Wassermann tubes, each containing batch of 5 islets and incubated with 1 mL KRH buffer supplemented with 0.5% bovine serum albumin and glucose 6 mmol/L, one of these groups was considered as normal control. In the remaining 3 groups, the following drugs were added: glimepiride (10 μmol/L), vanadyl sulfate (1 μmol/L) or combination of both glimepiride and vanadyl sulfate, respectively. All the tubes were covered and incubated at 37°C in a shaking water bath for 1 h, then the tubes were transferred into ice-bath, mixed with vortex mixer and aliquots of 0.5 mL were taken and kept frozen at -20°C for insulin determination.

Biochemical estimations:

In vivo experiments: Serum glucose level was estimated using glucose kit (spinreact, Spain) (Trinder, 1969) and expressed as mg/dL. Whereas, serum insulin was assayed using radioimmunoassay kits (Coat-a-Count kit -DPC, Los Angeles, CA, USA) (Mullner et al., 1991) and expressed as μIU/mL.

Liver glycogen was estimated (Kemp and Van Heijningen, 1954) and expressed as mg/g wet tissue.

Serum lipid peroxides level was estimated by determination of the level of Thiobarbituric Acid Reactive Substances (TBARS) that were measured as MDA (Mihara and Uchiyama, 1978) and expressed as nmol/mL.

Blood SOD was estimated using the pyrogallol method (Marklund and Marklund, 1974) and expressed as ug/mL.

Blood GSH was estimated according to the method of (Beutler et al., 1963) and expressed as mg %.

In vitro experiment: Insulin level was measured using radioimmunoassay kits (Coat-a-Count kit -DPC, Los Angeles, CA, USA) (Mullner et al., 1991) and expressed as μIU/mL.
Statistical analysis: The values of the measured parameters were presented as mean±S.E.M. Comparisons between different treatments were carried out using one way Analysis of Variance (ANOVA) followed by Tukey-Kramer as post ANOVA multiple comparisons test. Differences were considered statistically significant when p<0.05.

RESULTS

Effect of glimepiride, vanadyl sulfate or their combination on serum glucose and insulin levels in STZ-induced diabetic rats after two weeks of daily dose administration: STZ significantly increased serum glucose level and significantly reduced the serum insulin level in STZ-diabetic rats.

Glimepiride and vanadyl sulfate significantly reduced the serum glucose level to 54.77 and 57.35% of the diabetic control value, respectively. Combination of vanadyl sulfate and glimepiride did not significantly affect the hypoglycemic action of glimepiride (Fig. 1).

Glimepiride significantly increased the serum insulin level to 220.46% of the diabetic control value. However, vanadyl sulfate was unable to normalize the decreased serum insulin level induced by STZ. Concurrent administration of vanadyl sulfate and glimepiride, in the selected doses, could not improve the effect of glimepiride alone on serum insulin level in STZ-diabetic rats (Fig. 2).

Effect of glimepiride, vanadyl sulfate or their combination on liver glycogen content in STZ-induced diabetic rats after two weeks of daily dose administration: STZ significantly reduced liver glycogen content in diabetic control group. Glimepiride and vanadyl sulfate significantly increased liver glycogen content of diabetic rats to 195.26 and 177.299% of the diabetic control value, respectively. Combination of vanadyl sulfate and glimepiride, when given in the selected doses, did not significantly change the effect of glimepiride on liver glycogen content (Fig. 3).

Effect of glimepiride, vanadyl sulfate or their combination on oxidative stress biomarkers in STZ-induced diabetic rats after 2 weeks of daily dose administration: STZ significantly lowered the blood GSH level in diabetic rats. Glimepiride normalized the blood GSH level of diabetic rats to 81.49% of the normal control value. However, this value was still non-significant from that of the diabetic control group recording 150.05% of the diabetic control value. Vanadyl sulfate significantly elevated the blood GSH level of diabetic rats. Glimepiride normalized the blood GSH level of diabetic rats to 81.49% of the normal control value. However, this value was still non-significant from that of the diabetic control group recording 150.05% of the diabetic control value. Vanadyl sulfate significantly elevated the blood GSH level of diabetic rats.

Fig. 1: Effect of glimepiride, vanadyl sulfate or their combination on serum glucose level in STZ-induced diabetic rats after two weeks of daily dose administration

Diabetes was induced by a single injection of STZ (50 mg/kg, i.p.) in all groups except the normal control one, which received an equivalent volume of citrate buffer. Drug treatment was started 72 h after STZ administration once daily, for two successive weeks. Blood samples were collected 2 h after the last dose administration; N: 6-7 rats per group; Data were expressed as mean±S.E. of the mean; *: Significantly different from the normal control value at p<0.05; a: Significantly different from diabetic control value at p<0.05.
Fig. 2: Effect of glimepiride, vanadyl sulfate or their combination on serum insulin level in STZ-induced diabetic rats after 2 weeks of daily dose administration.

Diabetes was induced by a single injection of STZ (50 mg/kg, i.p.) in all groups except the normal control one, which received an equivalent volume of citrate buffer. Drug treatment was started 72 h after STZ administration once daily, for two successive weeks. Blood samples were collected 2 h after the last dose administration; N: 6-7 rats per group; Data were expressed as mean±S.E. of the mean; *: Significantly different from the normal control value at p<0.05; a: significantly different from diabetic control value at p<0.05; b: Significantly different from glimepiride value at p<0.05.

Fig. 3: Effect of glimepiride, vanadyl sulfate or their combination on liver glycogen content in STZ-induced diabetic rats after two weeks of daily dose administration.

Diabetes was induced by a single injection of STZ (50 mg/kg, i.p.) in all groups except the normal control one, which received an equivalent volume of citrate buffer. Drug treatment was started 72 h after STZ administration once daily, for two successive weeks. Liver was isolated 2 h after the last dose administration and homogenized in saline to be used for determination of glycogen content; N: 6-7 rats per group; Data were expressed as mean±S.E. of the mean; *: Significantly different from the normal control value at p<0.05; a: Significantly different from diabetic control value at p<0.05.
Fig. 4: Effect of glimepiride, vanadyl sulfate or their combination on (6 mmol/L) glucose-stimulated insulin secretion from isolated rat pancreatic islets.

Islets were isolated from non-fasting rats according to the collagenase digestion technique and pre-incubated in Krebs-Ringer-HEPES medium (KRH) at 37ºC for 30 min. After the pre-incubation period, batches of five islets were transferred to a medium containing 1 mL KRH buffer supplemented with 0.5% bovine serum albumin, glucose (6 mmol/L), and the specified drug. Islets were incubated at 37ºC in a shaking water bath for 1 h. After the incubation period, the medium was assayed for insulin content; N: 5-6 rats per group; Data were expressed as mean± S.E. of the mean; *: Significantly different from the control value at p<0.05; a: Significantly different from glimepiride value at p<0.05.

Table 1: Effect of glimepiride, vanadyl sulfate or their combination on oxidative stress biomarkers in STZ-induced diabetic rats after 2 weeks of daily dose administration

<table>
<thead>
<tr>
<th>Parameter/drugs &amp; doses</th>
<th>Oxidative stress biomarkers</th>
<th>( \text{Plasma GSH (mg %)} )</th>
<th>( \text{Serum TBARS (nmol/mL)} )</th>
<th>( \text{Plasma SOD (U/mL)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (citrate buffer and Tween 80)</td>
<td></td>
<td>38.52±1.52</td>
<td>2.11±0.15</td>
<td>27.47±1.57</td>
</tr>
<tr>
<td>Diabetic control (STZ, 50 mg/kg, i.p.)</td>
<td></td>
<td>20.92±1.54*</td>
<td>6.16±0.15*</td>
<td>12.70±1.80*</td>
</tr>
<tr>
<td>Glimepiride (10 mg/kg, p.o.)</td>
<td></td>
<td>31.39±2.59</td>
<td>3.04±0.179*</td>
<td>21.29±1.73*</td>
</tr>
<tr>
<td>Vanadyl sulfate ( \text{(VOSO}_4 \text{)} ) (15 mg/kg, i.p.)</td>
<td></td>
<td>31.19±2.13*</td>
<td>3.32±0.21*</td>
<td>21.29±1.74*</td>
</tr>
<tr>
<td>Glimepiride + VOSO(_4) (10 mg/kg,p.o.) + (15 mg/kg, i.p.)</td>
<td></td>
<td>34.35±2.25*</td>
<td>2.21±0.197*</td>
<td>24.37±1.24*</td>
</tr>
</tbody>
</table>

STZ induced a significant increase in serum TBARS level in diabetic control rats. Glimepiride and vanadyl sulfate significantly lowered serum TBARS level of diabetic rats to 49.35 and 53.896% as compared to diabetic control value, respectively. Combination of vanadyl sulfate and glimepiride had the same effect of glimepiride alone on serum TBARS level indicating there is no significant interaction between these two drugs (Table 1).

GSH level to 149.09% of the diabetic control value. It is evident that there was no significant interaction between vanadyl sulfate and glimepiride when given in the selected doses (Table 1).

STZ caused a significant reduction in superoxide dismutase level in the diabetic control rats. Glimepiride and vanadyl sulfate significantly increased superoxide dismutase value to 167.64 and 167.64% of the diabetic control value, respectively. Combination of vanadyl sulfate and glimepiride was not significantly different when compared to glimepiride monotherapy (Table 1).
Effect of glimepiride, vanadyl sulfate or their combination on (6 mmol/L) glucose-stimulated insulin secretion from isolated rat pancreatic islets: The insulin concentration of the control group was 5.02±0.28 µIU/h/islet.

Glimepiride significantly increased insulin secretion from rat pancreatic islets in presence of 6 mmol/L glucose. However, vanadyl sulfate did not significantly affect insulin secretion from rat pancreatic islets in presence of 6 mmol/L glucose. No interaction has been observed when vanadyl sulfate is given with glimepiride. Combination of vanadyl sulfate with glimepiride did not significantly affect glimepiride-induced increase in insulin secretion from rat pancreatic islets (Fig. 4).

**DISCUSSION**

In the present study, diabetes was induced by a single injection of streptozotocin (50 mg/kg, i.p.) which was reported to increase blood glucose level and decrease insulin sensitivity index, that are the main characteristics of type II diabetes mellitus (Niu et al., 2007).

Findings of the current study revealed that glimepiride (10 mg/kg) significantly reduced the serum glucose level of STZ-induced diabetic rats after two weeks of daily dose administration. This result is in accordance with that of (Mir et al., 2008; Hsu et al., 2009; Mowla et al., 2009).

In addition, glimepiride significantly elevated serum insulin level in STZ-diabetic rats. This result is in total agreement with that of (Rosenstock et al., 1996; Korytkowski et al., 2002; Hsu et al., 2009). The aforementioned in vitro results were also supported by the in vivo results that glimepiride (10 µmol/L) significantly increased glucose (6 mmol/L)-stimulated insulin secretion from isolated rat pancreatic islets. Similarly, (Hsu et al., 2009) found that glimepiride stimulated insulin release from rat pancreatic islets.

Depending on the findings of the present study, it could be suggested that the hypoglycemic effect of glimepiride was attributed to its stimulation of insulin secretion. This explanation is in accordance with that given by (Philipson and Steiner, 1995; Fuhlendorff et al., 1998; Muller, 2005) who found that glimepiride binds to sulfonylurea receptors on β-cells leading to blocking of K _ATP_ channels, opening of voltage-gated calcium channels and increase in Ca²⁺ influx leading to insulin release from pancreatic β-cells.

In contrast to our results (Duckworth et al., 1972; Olefşky and Reaven, 1976; Beck-Nielsen et al., 1979; See et al., 2003) observed that glimepiride has hypoglycemic action without significant effect on plasma insulin level, indicating that glimepiride has, in addition, an extrapancreatic activity which includes both insulin-mimetic and insulin-sensitizing activity (Muller, 2005).

Results of the current study revealed that vanadyl sulfate (15 mg/kg, i.p.) showed a significant reduction in the serum glucose level of STZ-induced diabetic rats. Similar results have been reported by (Bendayan and Gingras, 1989; Mongold et al., 1990; Dai et al., 1994; Poucheret et al., 1995; Ray et al., 2004; Bolkent et al., 2005; Rashwan and Al-Firdous, 2011).

However, according to this study the improvement in the serum glucose level induced by vanadyl sulfate was not accompanied by increase in serum insulin level in STZ-induced diabetic rats. Results of the present study confirm the study of (Brichard et al., 1989; Poucheret et al., 1995; Cadene et al., 1996; De Tata et al., 2000).

The effect of vanadyl sulfate on in vivo insulin secretion of this study is also confirmed by the in vitro study (isolated rat pancreatic islets), where data of the present investigation revealed that vanadyl sulfate (1 µmol/L) inhibited glucose (6 mmol/L)-stimulated insulin secretion from isolated rat pancreatic islets. These results are consistent with the data given by (Voss et al., 1992).

Depending on the aforementioned findings of this study, it could be suggested that the hypoglycemic action vanadyl sulfate is most probably attributed to its insulin-mimetic effects, which has been observed by (Siddiqui et al., 2005; Wilsey et al., 2006) or to its insulin-sensitizing effect as reported by (Cohen et al., 1995; Fantus and Tsiani, 1998; Cam et al., 1999; Verma et al., 1998). This insulin-sensitizing action of vanadium might be attributed to its inhibitory effect of Protein Tyrosine Phosphatases (PTPs) (Sakurai et al., 2006) particularly PTP1B, whose inhibition will lead to stimulation of insulin receptors (Ramachandran et al., 1992; Ahmad et al., 1995; Seely et al., 1996; Bandyopadhyay et al., 1997; Wang et al., 2001).

Concerning the liver glycogen content, results of this study have demonstrated that the diabetic control group showed a significant decrease in liver glycogen content after STZ injection. Similar results were obtained by (Rashwan and Al-Firdous, 2011).

Glimepiride, in a dose of 10 mg/kg, significantly increased liver glycogen content of STZ-diabetic rats. This finding is in full agreement with other studies (Muller and Wied, 1993; Muller and Geisen, 1996; Muller, 2000; Haupt et al., 2002; Mori et al., 2008). These results suggest that glimepiride stimulates glycogenesis and this confirms the study of (See et al., 2003).
This result can be explained by Muller (2000) who reported that glimepiride activates insulin receptors and thereby it can possibly stimulate insulin-induced glycogen synthesis. In addition, (Olbrich et al., 1999; Reimann et al., 2000) found that sulfonylurea’s inhibit K\textsubscript{ATP} channels and thereby control the intracellular Ca concentrations. This regulation of intracellular Ca content can affect the insulin-signaling cascade and thereby stimulates glycogen synthesis (Haupt et al., 2002).

Vanadyl sulfate normalized the liver glycogen content decreased by STZ. These results are in parallel with the data obtained by Tolman et al. (1979), Niu et al. (2007) and Rashwan and Al-Firdous (2011).

The most likely explanation for this action is that vanadium activates the glucose-sensing enzyme, glucokinase, in the liver and pancreas. This explanation is in agreement with (Niu et al., 2007). This enzyme has been reported to stimulate glucose uptake and glycogen synthesis in the liver and thereby decrease the blood glucose level (Efanov et al., 2005).

Data of the current study revealed that STZ caused a significant increase in serum TBARS level accompanied by a significant reduction in blood GSH and SOD levels. These results are in harmony with that of (El-Missiry et al., 2004; Preet et al., 2005; Siddiqui et al., 2005; Shukla et al., 2007) for TBARS; (Yarat et al., 2001; Bolkent et al., 2005; Preet et al., 2005; Kakadiya et al., 2010) for GSH and (Siddiqui et al., 2005; Shukla et al., 2007; Kakadiya et al., 2010) for SOD.

Findings of the present investigation showed that glimepiride significantly lowered serum TBARS level and significantly increased SOD and GSH levels in STZ-diabetic rats. Similar results have been reported by (Krauss et al., 2003; Kakadiya et al., 2010; Kakadiya and Shah, 2011) for TBARS; (Kakadiya et al., 2010; Kakadiya and Shah, 2011) for GSH and (Krauss et al., 2003; Rabbani et al., 2009; Kakadiya et al., 2010; Kakadiya and Shah, 2011) for SOD.

The present observations suggest that glimepiride possesses antioxidant activity against the STZ-induced oxidative stress. This suggestion is in accordance with that of (Rabbani et al., 2009). This antioxidant effect of glimepiride may be attributed to its activation of the redox sensitive transcription factor NF (Kappa) B, activation of antioxidant enzymes such as SOD which is responsible for dismutation of superoxide ion into oxygen and hydrogen peroxide, thus protecting the cell from damage caused by superoxide activity (Kono, 1978; Valko et al., 2007) and to its free radical quenching properties, which leads to an increase in the number of β-cells in the islets of Langerhans in glimepiride-treated diabetic animals (Schiekofor et al., 2003).

Vanadyl sulfate returned the level of serum TBARS to the normal value and significantly elevated the blood GSH and SOD levels as compared to the diabetic control value. These results are in accordance with (Siddiqui et al., 2005; Shukla et al., 2007) for TBARS; (Bolkent et al., 2005; Preet et al., 2005) for GSH and (Siddiqui et al., 2005; Shukla et al., 2007) for SOD.

The most probable explanation for these antioxidant effects of vanadyl sulfate may be attributed to its ability to normalize the decreased activity of Na\textsuperscript{+}/K\textsuperscript{+} ATPase, increased lipid peroxides and altered membrane fluidity caused by diabetes, which in turn will lead to a decrease in the production of free radicals, lipid peroxides and restoring the antioxidant enzymes activity (Siddiqui et al., 2005).

Finally, according to the findings of the present study, it could be stated that there is no significant interaction between vanadyl sulfate and glimepiride when used in combination on any of the aforementioned parameters. It follows that the two drugs can be taken together safely without fear of any serious reactions. The absence of additive action between the two drugs observed in this study may be attributed to the use of doses which give maximal response, thus no potentiating of action was observed.

REFERENCES


