EFFECT OF VANADATE ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYME ACTIVITIES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

It has been suggested that, in diabetes, oxidative stress plays a role in the pathogenesis of vascular complications, both microvascular and macrovascular (295) and an early markers of such damage in the development of an endothelial dysfunction (295,296). However, the role of oxidative stress in diabetes is questioned by the results of intervention studies with antioxidants, which are elusive or unsuccessful (298).

Diabetes mellitus has been known to be an increased state of free radical formation. Oxygen free radicals (OFRs) have been implicated in the pathogenesis of diabetes mellitus (295). The persistent hyper-glycaemia in diabetes causes increased production of oxygen free radicals (Superoxide, peroxide and hydroxy radical) through autooxidation of glucose (97) and also by non-enzymatic protein glycation (296). OFRs exert their cytotoxic effects on membrane phospholipids, resulting in the formation of malondialdehyde (MDA). Peroxidation of membranes increases its fluidity and permeability with loss of membrane integrity (297,298). The levels of the reactive oxygen species are controlled by antioxidant enzymes namely superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione peroxidase (GPx; EC 1.11.1.9) protecting cells and tissues against oxidative injury (299).
Increase in levels of OFRs could be due to their increased production and/or decreased destruction. Oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the development of vascular disease in diabetes (97,98).

Much of the experimental evidence suggests that diabetes and hyperlipidaemia alone are not sufficient to induce vascular disease but oxidative stress may be an important and independent risk factor in the development of vascular disease (97). Apart from increased non-enzymatic glycosylation and auto oxidative glycosylation, metabolic stress resulting from changes in status of antioxidant defense systems could lead to oxidative stress in diabetes. Marked changes in antioxidant enzyme activities and tissue glutathione concentrations have been reported in diabetes (300,301). There is evidence that there are alterations in free radical metabolism during diabetes in various tissues, but the alterations are quite heterogenous.

Recent studies have shown that vanadate is a potent insulin mimetic agent and promotes glucose uptake, (123) glucose oxidation (111) and activation of glycogen synthase in rat liver (140) and adipocytes. Oral administration of vanadate to streptozotocin induced diabetic rats, in our study, has resulted in the normalisation of blood glucose and also restoration of the activity of key enzymes of glycolysis (Hexokinase) and gluconeogenesis (Glucose-6-phosphatase and Fructose 1-6-bis phosphatase) to normal levels which has been presented in the
third chapter of this thesis. Wohaleb and Godin (301) demonstrated previously that insulin treatment of diabetic rats resulted in almost complete reversal of all the foregoing alterations in tissue antioxidant status. Hence, an antioxidant role was presumed for vanadate, in view of its insulin-mimetic properties of correcting various metabolic enzymes.

The present study was, therefore, taken up to examine whether, like insulin, vanadate treatment can normalize the lipid peroxidation and the disturbed antioxidant status in experimental diabetes.

**Experimental Design**

The experimental animals were divided into four groups each consisting of six animals. Group 1 consisted of normal rats fed on the commercial pellet diet and given only saline (0.9% NaCl). Group 2 rats were treated with SOV alone to test whether it had any side effects. This group was fed on commercial pellet diet and saline with SOV. Group 3 rats were used as a diabetic control group by administering STZ by ip injection after 24 hrs of fasting. These animals were fed on commercial pellet diet and saline. Group 4 rats were STZ induced diabetic, which were treated with SOV (SOV 0.6mg/ml of H₂O). They received commercial pellet diet and saline with SOV. After 5 weeks of SOV treatment the animals were fasted over night and then killed under ether anaesthesia. Blood was collected for determining blood glucose, HbA₁ and lipid peroxides. Hemolysate was prepared and used for the
measurement of antioxidant enzyme activities. The tissues (Liver and Kidney) were removed and homogenised in 10 volumes of 50mM phosphate buffer. Supernatant was used for measuring antioxidant enzyme activities and lipid peroxides (TBARS).

RESULTS

The changes in the body weight, plasma glucose, and insulin for the 4 groups of rats are given in table-13. The body weights of diabetic animals were lowered compared to normal controls, but treatment with vanadate significantly increased the body weight in diabetic rats. Plasma glucose of diabetic rats was higher than in controls, while it was near to normal levels in vanadate treated diabetic rats. The plasma insulin was decreased in diabetic rats, which was not altered by vanadate treatment.

The level of plasma TBARS and erythrocyte antioxidant enzyme activities (CAT, SOD, GPx) in the 4 groups of rats are given in table 14. There was a significant increase (P<0.01) in TBARS of plasma in diabetic rats as compared to those in controls, whereas in vanadate treated diabetic rats the TBARS levels were decreased when compared to those of diabetic rats but this decrease was not nearer to the level of TBARS in controls. The erythrocyte catalase activity of diabetic rats was significantly increased (p<0.001) in diabetic rats compared to that of control rats. After treatment with vanadate in diabetic rats, the activity of catalase was significantly decreased (p<0.001) to the level of normal controls.
Table 13: Changes in body weight, blood glucose, glycated haemoglobin (HbA₁), plasma insulin in the control and experimental groups of rats (Means ±SD of six rats in each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight change (g/week)</th>
<th>Blood Glucose (mg/dl)</th>
<th>HbA₁ (%)</th>
<th>Plasma Insulin (pmoles/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>+30.5± 0.7</td>
<td>80.23±8.16</td>
<td>7.6±0.85</td>
<td>238.2±30.73</td>
</tr>
<tr>
<td>Group 2</td>
<td>+27.7±0.8</td>
<td>78.88±7.45</td>
<td>7.3±0.9</td>
<td>224±27.45</td>
</tr>
<tr>
<td>Group 3</td>
<td>-15.7±0.5*</td>
<td>315.74±22.5*</td>
<td>14.7±2.02*</td>
<td>74.8±6.7*</td>
</tr>
<tr>
<td>Group 4</td>
<td>+24.8±0.55†</td>
<td>105.11±8.07</td>
<td>8.9±1.8†</td>
<td>105.7±5.15†</td>
</tr>
</tbody>
</table>

*P<0.001 Compared to controls
†P<0.001 Compared to untreated diabetic rats

Group-1: Control normal rats
Group-2: Rats treated with SOV alone
Group-3: STZ induced diabetic rats
Group-4: STZ + SOV treated rats
Table -14: Plasma TBARS levels and erythrocyte antioxidant enzyme activities in the 4 groups of rats (Means ± SD of six rats each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxides (µ moles MDA/L)</th>
<th>Catalase (KU/g Hb)</th>
<th>Glutathione peroxidase (U/mg Hb)</th>
<th>Superoxide dismutase (U/mg Hb/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.423±0.05</td>
<td>28.53±4.3</td>
<td>0.361±0.06</td>
<td>7.25±0.37</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.499±0.02</td>
<td>34.44±3.0</td>
<td>0.325±0.05</td>
<td>6.65±0.77</td>
</tr>
<tr>
<td>Group 3</td>
<td>1.01±0.47*</td>
<td>53.45±6.1*</td>
<td>0.157±0.02*</td>
<td>2.34±0.52*</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.686±0.38*</td>
<td>30.18±3.5†</td>
<td>0.295±0.05†</td>
<td>6.58±0.71†</td>
</tr>
</tbody>
</table>

*P<0.001 Compared to controls
†P<0.001 Compared to untreated diabetic rats

Group -1: Control normal rats
Group -2: Rats treated with SOV alone
Group -3: STZ induced diabetic rats
Group -4: STZ+SOV treated rats
The activities of other two antioxidant enzymes GPₓ and SOD were significantly lower in the RBC of diabetic rats than those in normal control rats. Vanadate treatment in the diabetic rats resulted in an increase in the activities of SOD and GPₓ (p<0.001).

Table-15 depicts the TBARS levels and antioxidant enzymes in livers and kidneys of four groups of rats. The level of TBARS in diabetic rats was significantly higher (P<0.001) in both the tissues (liver and kidney) compared to those in controls, and treatment with vanadate normalized the TBAR levels in both liver and kidney of diabetic rats. The Catalase activities in liver and kidney of diabetic rats were significantly higher (P<0.001) than those in controls. In the vanadate treated, diabetic rats the activities of catalase in both liver and kidney were significantly lowered (P<0.001)) to the level of normal rats.

The GPₓ and SOD activities from kidney and liver of STZ induced diabetic rats were significantly decreased (P<0.001) when compared to those in controls. Upon vanadate treatment the activities of both the enzymes in both tissues were restored (P<0.001) near to normal levels. Treatment with vanadate did not alter any of these enzyme activities in controls.
Table -15: TBARS levels and Antioxidant enzyme activities in the livers and kidneys of different experimental groups (Means ± SD of six rats each group)

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipid peroxidation (μ moles MDA/ mg protein)</td>
<td>Catalase (KU/mg protein)</td>
</tr>
<tr>
<td>Group 1</td>
<td>2.85±0.69</td>
<td>0.232±0.03</td>
</tr>
<tr>
<td>Group 2</td>
<td>3.93±0.89</td>
<td>0.264±0.04</td>
</tr>
<tr>
<td>Group 3</td>
<td>6.12±1.6*</td>
<td>0.420±0.05*</td>
</tr>
<tr>
<td>Group 4</td>
<td>5.65±1.1†</td>
<td>0.265±0.04†</td>
</tr>
</tbody>
</table>

*P<0.001 compared to controls  
†P<0.001 compared to untreated diabetic rats

Group - 1: Control normal rats  
Group - 2: Rats treated with SOV alone  
Group - 3: STZ induced diabetic rats  
Group - 4: STZ+SOV treated rats

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DISCUSSION

The mechanism of the insulin-mimetic effects of vanadium compound is not completely understood. Vanadium is now considered as an essential nutritional trace element and has therapeutic value in pharmacological doses, but toxic in excess and causes stimulation of $H_2O_2$ production and lipid peroxidation (303). Our studies, and those of others, have demonstrated that Vanadate mimics many of the actions of insulin and its oral administration improves the altered glucose homeostasis and other metabolic disorders of the diabetic state (123, 111, 113, 140, 302, 304, 305). There is now ample evidence to suggest that, in certain pathological states like diabetes, the increased production and ineffective scavenging of toxic reactive oxygen species may play a vital role in determining tissue injury (300, 296, 306, 301). Diabetes causes a depression in overall liver and kidney antioxidant status making them vulnerable to oxygen radical attack which may cause oxidative damage to membranes and alterations in subcellular organelle structural and functional integrity (301).

In the present study the levels of TBARS were increased in both blood and tissues (Liver and Kidney) of diabetic rats. Increased levels of TBARS were reported by Rakesh et al (306) in heart, pancreas and blood of streptozotocin induced diabetic rats. In one of our studies an increase in the TBARS levels of plasma in human type 2 diabetic patients was observed (307), which is mentioned in the first chapter of this thesis. The increased levels of TBARS suggest an increased levels of reactive oxygen species which could be due to their increased production or decreased destruction. Increase in ROS levels in diabetes could be
due to increase in blood glucose levels. Glucose can increase ROS through autooxidation and through non enzymatic protein glycation (97,296).

Wolff and Dean (101) suggested that non enzymatic protein glycation a mechanism proposed early on to account for glucose cytotoxicity (102), was dependent on ROS (superoxide as hydroxyl) formation through transition metal catalysed glucose autooxidation. Research in several laboratories has indicated that hyperglycaemia activates several major well-characterized biochemical pathways that play a significant role in the etiology of diabetic complications.

In the present study the increased catalase activity could be due to higher production of $H_2O_2$. Catalase has been reported to be responsible for detoxification of $H_2O_2$. Hypoinsulinaemia as in STZ-Induced diabetes increases the activity of enzyme fatty acyl CO-A oxidase that initiates $\beta$-oxidation of fatty acids resulting in production of $H_2O_2$ (308). $H_2O_2$ is not only toxic to cells but also permeable to cell membranes. The increased CAT activity in diabetic rats could be a compensatory mechanism in response to increased oxidative stress. The increase in CAT activity observed in blood and liver of diabetic rats is in agreement with that reported by Rakesh et al (306) and in disagreement with that reported by Saxena et al (309). The increased CAT activity in diabetic rats was reversed to normal level by vanadate treatment.

GPx, a selenium - dependent enzyme, reduces hydrogen peroxide and organic peroxides and leads to the oxidation of glutathione (GSH).
Along with the other antioxidant enzymes, GPx protects cells and tissues from damage caused by reactive oxygen species by helping to maintain balance between pro-oxidant and antioxidant forces (310,311).

GPx has a key role in enzymatic defence systems and acts on peroxides (H₂O₂, Lipid or Organic peroxides) to remove them. SOD has a key role in enzymatic defense systems and acts on O₂⁻ and hydrogen peroxide. In the present study, it was observed that vanadate treatment could effectively normalize the GPx and SOD levels. In diabetic rats, the activities of GPx and SOD both in blood and tissues were significantly decreased which is in agreement with the report of Prince et al (312) suggesting that there was an increased oxidative stress during diabetes (involving increased O₂⁻ and H₂O₂ production). The other groups have reported elevated levels of GPx and SOD in hyperglycaemic rats (313, 314). Sekar et al (315) have reported restoration of catalase in vanadate treated diabetic rats, while Saxena et al (309) have reported normalization of glutathione peroxidase and Mn-superoxide dismutase activities in vanadate treated diabetic rats. The increase in oxygen radicals during diabetes may be due to decreased activities of GPx and SOD, which are involved in scavenging the free radicals. H₂O₂ is known to inactivate SOD (316). Decrease in SOD activity can protect catalase and glutathione peroxidase against inactivation by O₂⁻ anion. The increase in TBARS associated with the increase in catalase and decrease in glutathione peroxidase and superoxide dismutase activities could be due to increased production of H₂O₂ and other oxygen free radicals.
It has recently been suggested that diabetic subjects with complications may have a defective cellular antioxidant response against the oxidative stress generated by hyperglycaemia, which can predispose the patient to organ damage (317). New insights into the mechanisms leading to the generation of oxidative stress in diabetes are now available. Presently these findings have led to the discovery and to the evaluation of new antioxidant molecules, such as SOD and catalase mimetics, that hopefully may inhibit at an early stage, the mechanism leading to diabetic complications.

In conclusion, the present investigation indicates that vanadate therapy is effective in normalising the altered antioxidant enzyme activities and TBARS levels. However, further studies are needed to explore the mechanism of action of vanadate on these antioxidant defence systems.