Effect of chronic treatment with Bis(maltolato)oxovanadium (IV) in rat model of non-insulin-dependent-diabetes

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Effect of chronic treatment with Bis(maltolato)oxovanadium (IV) (BMOV) was studied in streptozotocin (STZ)-induced neonatal non-insulin-dependent-diabetic (NIDDM) rats. Intraperitoneal injection of STZ (90 mg kg\(^{-1}\)) in Wistar rat pups (day 2 old) produced mild hyperglycemia, impaired glucose tolerance and insulin resistance at the age of 3 months. Treatment with BMOV (0.23 mM kg\(^{-1}\)) in drinking water for 6 weeks produced a significant decrease in elevated serum glucose levels without any significant change in serum insulin levels in diabetic rats. BMOV treatment significantly decreased integrated area under the glucose curve without any significant change in integrated area under the insulin curve indicating improved glucose tolerance. Treatment also significantly increased 1\(_{IRV}\) value of diabetic rats indicating increased insulin sensitivity. BMOV treatment significantly reduced hypercholesterolemia in diabetic rats. Treatment also significantly decreased serum triglyceride levels in both diabetic and non-diabetic rats. The data suggest that chronic BMOV treatment improves glucose and lipid homeostasis. These effects appear to be due to the insulin sensitizing action of vanadium.

Vanadium, a group Va transition element was first reported to produce glucose lowering and cardioprotective effects in streptozotocin (STZ)-diabetic rats by Heyliger et al.\(^1\). Since then a great deal of work with vanadium as a potential anti-diabetic therapy has been done. Insulin-mimetic actions of vanadium have been well documented in several in vitro and in vivo models of Type I diabetes including chemically STZ-induced diabetes in rats\(^7-10\) and the genetically predisposed BB Wistar rats\(^11,12\). However, there are drawbacks to vanadate because it is poorly absorbed from the gastrointestinal tract and the doses of vanadate required for its anti-diabetic effect are close to the toxic level\(^13,14\). Vanadyl sulphate is less toxic than vanadate but is also not well absorbed from the gastrointestinal tract. Complexation of vanadyl ion within an organic matrix has been used to increase the potency of vanadium with respect to the insulin mimetic activity\(^15-18\). Bis(maltolato)oxovanadium (IV) (BMOV)\(^16\), a coordination complex of vanadyl and maltol has been shown to be two to three times more potent glucose lowering agent than inorganic vanadium\(^19\).

Since one of the mechanisms of action postulated for vanadium has been the sensitization of tissues to actions of insulin, vanadium may be useful as an oral treatment in non-insulin dependent diabetes mellitus (NIDDM) where there is typically some residual \(\beta\)-cell function. Vanadium in few studies has been shown to improve glucose homeostasis in genetically obese, hyperinsulinaemic and insulin-resistant rats and mice exhibiting some of the characteristics of Type II diabetes\(^20-23\). An experimental model of Type II diabetes mellitus developed by Portha et al.\(^24\) and Bonner-Weir et al.\(^25\) involves injection of STZ (90 mg kg\(^{-1}\), i.p.) into two day old neonatal rats. Studies from various other laboratories including that of ours have further shown that neonatally-treated rats in adulthood display the typical characteristics of NIDDM\(^26-29\). The present study was undertaken to study the effect of chronic treatment with BMOV on glucose homeostasis and insulin sensitivity in neonatal-STZ NIDDM rats.

Materials and Methods

Animals—Wistar albino rats from an inbred colony were bred under well-controlled conditions of temperature (22° ± 2°C), humidity (55 ± 5%) and 12 hr/12 hr light-dark cycle. Conventional laboratory diet and tap water were provided ad libitum. Two-day-old male Wistar neonates were injected intraperitoneally with 90 mg kg\(^{-1}\) STZ (Sigma, USA) in 0.9% sodium chloride solution. Control neonates received equivalent amount of isotonic saline alone. The neonates
were left with their own mothers and weaned at 4 weeks of age. Twelve weeks after the injection of STZ, animals were checked for fasting glucose levels. The animals showing fasting glucose levels >140 mg dl^-1 were considered as diabetic. The experimental animals were divided into four groups as (i) non-diabetic control (ii) non-diabetic treated with BMOV (iii) diabetic control (iv) diabetic treated with BMOV.

Treatment protocol—BMOV was dissolved in water and administered at a concentration of 0.75 mg ml^-1 (0.23 mM kg^-1) ad libitum in the drinking water for 6 weeks. Changes in body weight, food and water consumption were monitored regularly. Blood samples were collected from 8 hr fasted animals under light ether anesthesia at the end of 6 weeks BMOV treatment by nicking the tip of tail. Serum was separated and analyzed for glucose, cholesterol and triglyceride using diagnostic reagent kits (Bayer Diagnostics India Ltd). Serum insulin, T₃, T₄ were estimated by radioimmunoassay technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai.

Oral glucose tolerance test (OGTT)—At the end of 6 weeks of treatment, oral glucose tolerance test was performed³⁰ wherein 8 hr fasted animals were administered orally with 1.5 g kg^-1 of glucose. Blood samples were collected from the tail vein using catheter before i.e. 0 and 15, 30, 60 and 120 min after oral glucose administration. Serum was separated and analyzed for glucose, cholesterol and triglyceride using diagnostic reagent kits (Bayer Diagnostics India Ltd). Serum insulin, T₃, T₄ were estimated by radioimmunoassay technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai.

Insulin Tolerance Test—Insulin sensitivity was studied by insulin tolerance test as per the method described by Alford et al.³¹. 8 hr fasted animals were injected intravenously with 0.2U 100g^-1 of purified porcine insulin (Actrapid, Novo Nordisk Pharma India Ltd). Blood samples were collected from the tail vein before i.e. 0 and 5, 10, 20 and 30 min after insulin administration as described earlier. Serum was separated and analyzed for glucose, Kᵦᵣᵣ, as an index of insulin-mediated glucose metabolism, was calculated using the formula given by Lundbaek³²:

\[ Kᵦᵣᵣ = \frac{0.693 \times 100}{t_{½}} \]

where \(t_{½}\) represents the half life of plasma glucose decay, obtained by plotting plasma glucose concentration versus time on semilogarithmic graph paper.

Statistical analysis—Results are presented as mean ± SE. Data were analyzed by one-way ANOVA followed by Tukey’s multivariance test. Differences were considered to be significant if \(P < 0.05\).

Results

General characteristics of experimental animals—Body weight in the diabetic group was not significantly different from that in the non-diabetic control group. Treatment with BMOV did not produce any significant effect on body weights of the non-diabetic or diabetic treated rats. There was no significant difference in the food and water consumption of diabetic control as well as BMOV treated non-diabetic or diabetic rats compared to the control rats (Table 1).

Effects of BMOV treatment on serum glucose and insulin levels—Serum glucose levels in the diabetic rats were significantly higher compared to control rats. However serum insulin levels of diabetic rats were not significantly different from that of control (Table 2). Chronic BMOV treatment significantly reduced serum glucose levels without any significant change in serum insulin in diabetic rats. Treatment did

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control treated</th>
<th>NIDDM Control</th>
<th>NIDDM treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g/rat)</td>
<td>102±14</td>
<td>103±15</td>
<td>110±20</td>
<td>94±11</td>
</tr>
<tr>
<td>Food consumption (g/rat/day)</td>
<td>26±7.2</td>
<td>23±5.2</td>
<td>31±5.3</td>
<td>33±3.5</td>
</tr>
<tr>
<td>Water consumption (ml/rat/day)</td>
<td>20±4</td>
<td>19±2</td>
<td>18±2</td>
<td>22±5</td>
</tr>
</tbody>
</table>

Table 1—Effect of chronic BMOV treatment on general characteristics of animals at the end of 6 weeks
[Values are mean ± SE of 6 observations]
not produce any significant change in serum glucose or insulin levels in non-diabetic rats (Table 2).

**Effects of BMOV treatment on serum cholesterol and triglyceride levels**—Type II diabetic rats showed significantly higher cholesterol levels compared to control rats. Serum triglyceride levels of diabetic rats were not significantly different from that of control (Table 2). BMOV treatment produced significant decrease in serum cholesterol and triglyceride levels in diabetic as well as non-diabetic control rats at the end of 6 weeks (Table 2).

**Effects of BMOV treatment on serum T3 and T4 levels**—Serum T3 and T4 levels of control and diabetic group animals were not significantly different from each other and BMOV treatment did not produce any significant alteration in serum T3 and T4 levels of the treated animals (Table 2).

**Glucose tolerance**—Fasting blood glucose levels in diabetic rats were significantly higher than control rats. After glucose load, the serum glucose levels in diabetic group were significantly higher over a period of 120 min compared to non-diabetic control group. There was no decline in serum glucose levels in diabetic control group even after 120 min of glucose challenge as opposed to the non-diabetic control group, which showed a decline in serum glucose levels after 30 min of glucose loading. The fasting serum glucose levels of diabetic rats treated with BMOV were significantly lower than diabetic control group and unlike the diabetic control group, glucose levels started declining after 60 min of glucose challenge. There was no significant difference between the serum glucose levels of non-diabetic control treated with BMOV and non-diabetic control

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**Table 2**—Effect of chronic BMOV treatment on various biochemical parameters
[Values are mean ± SE of 6 observations]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Fasting Glucose (mg d⁻¹)</td>
<td>89.6 ± 6.2</td>
</tr>
<tr>
<td>Insulin (μU ml⁻¹)</td>
<td>47.2 ± 4.2</td>
</tr>
<tr>
<td>Total Cholesterol (mg dl⁻¹)</td>
<td>69.2 ± 2.5</td>
</tr>
<tr>
<td>Triglyceride (mg dl⁻¹)</td>
<td>65.3 ± 3.8</td>
</tr>
<tr>
<td>T3 (ng ml⁻¹)</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>T4 (ng ml⁻¹)</td>
<td>198.0 ± 7.1</td>
</tr>
</tbody>
</table>

* Significantly different from non-diabetic control (P < 0.05); † Significantly different from diabetic control (P < 0.05)

**Table 3**—Effect of chronic BMOV treatment on glucose tolerance and insulin sensitivity
[Values are mean ± SE of 6 observations]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>AUCg</td>
<td>18917 ± 634</td>
</tr>
<tr>
<td>AUCI</td>
<td>7627 ± 268</td>
</tr>
<tr>
<td>KITT</td>
<td>4.2 ± 0.43</td>
</tr>
</tbody>
</table>

* Significantly different from non-diabetic control (P < 0.05); † Significantly different from diabetic control (P < 0.05).
Fig. 1—Effect of chronic BMOV treatment on serum glucose (A) and insulin (B) concentrations in fasted anaesthetized control (■), control treated (●), diabetic control (▲) and diabetic treated (●) rats during oral glucose tolerance test. Values are mean ± SE of 6 observations. * P < 0.05, significantly different from non-diabetic control. ** P < 0.05, significantly different from diabetic control.

Fasting serum insulin levels of diabetic rats were not significantly different from non-diabetic control group. The peak serum insulin levels for all the groups occurred at 30 min, followed by a decline towards normal levels by 120 min after oral glucose challenge (Fig. 1). There was no significant difference in the insulin response of diabetic control, non-diabetic control treated and diabetic treated groups to glucose load compared to non-diabetic control group (Fig. 1).

The integrated mean area under the plasma glucose curve over 120 min [AUC$_{0-120min}$] of control treated group was not significantly different from non-diabetic control. Mean AUC$_{0-120min}$ was significantly greater in diabetic control relative to non-diabetic control. Treatment with BMOV produced significant
reduction in $AUC_{g(0-120min)}$ of diabetic rats compared to diabetic control (Table 3). The integrated insulin response [$AUC_{g(0-120min)}$] of diabetic as well as non-diabetic and diabetic rats treated with BMOV was not significantly different from non-diabetic control group (Table 3).

Insulin sensitivity—Insulin sensitivity as measured by the glucose disposal rate ($K_{ITT}$) was significantly lower in diabetic animals compared to control animals. $K_{ITT}$ value in non-diabetic rats treated with BMOV was not significantly different from that of control rats. $K_{ITT}$ value in BMOV treated diabetic rats was significantly higher than that of diabetic control rats (Table 3).

Discussion

Previous studies have reported that neonatal administration of streptozotocin (STZ), an effective pancreatic β cell toxin, results in the development of non-insulin dependent diabetes mellitus in the adult state. It is reported that STZ (90 mg/kg) when given intraperitoneally to 2-day old male rat pups produces transient hyperglycemia that lasts for 2-4 days followed by near normal glucose levels until about 6 week of age, whereupon frank chronic hyperglycemia develops with plasma glucose concentrations usually ranging between 200-350 mg dl$^{-1}$. In the present investigation, male Wistar rat pups injected on 2$^{nd}$ day with STZ (90 mg kg$^{-1}$, i.p.) showed mild hyperglycemia and glucose intolerance at 12 week of age. The treatment normalized increased serum glucose without any significant change in serum insulin levels in diabetic animals indicating improvement in glucose homeostasis. The decrease in serum glucose levels without a concomitant decrease in serum insulin levels is indicative of insulin sensitizing action of vanadium. It seems that either by replacing or potentiating the actions of endogenous insulin, vanadium causes a negative feedback inhibition of insulin release in diabetic rats.

When subjected to OGTT, the amount of insulin released in response to a glucose challenge was not significantly different in diabetic group despite normal basal serum insulin levels. Combination of glucose intolerance in face of normal or hyperinsulinemia clearly indicated insulin resistant state. AUCi of diabetic rats treated with BMOV was also not altered, however, AUCg was significantly lower compared to diabetic animals supporting insulin sensitizing effect of vanadium in insulin resistant type II diabetic rats. Treatment with BMOV did not significantly alter either AUCi or AUCg of non-diabetic animals. Vanadium has been postulated to be an insulin enhancer as opposed to insulin-mimetic in vivo. The insulin mimetic effects attributed to vanadium in vitro have been demonstrated to occur at higher concentrations ($10^{-4}$ - $10^{-5}$ M) than used for in vivo administration.

Results of insulin tolerance test (ITT) as per Alford et al. further support the observations of insulin resistant state of diabetic animals and insulin sensitizing action of vanadium. The value of $K_{ITT}$ for diabetic rats was found to be significantly lower compared to the non-diabetic control rats indicating insulin resistance in these animals. Treatment with BMOV produced a significant increase in the value of $K_{ITT}$ of diabetic rats indicating improved insulin sensitivity.

Bonner-Weir et al. have reported retarded growth of STZ-treated animals compared to control animals. However, in the present investigation, the body weight, food and water consumption of diabetic and non-diabetic animals was not significantly different from each other. BMOV treatment did not produce any significant effect on the general features of experimental animals mentioned earlier indicating lack of toxic effects of chronic BMOV administration. It is also clear that effects observed due to the chronic BMOV treatment are independent of the effects on body weight or food-water consumption.

An intimate relationship between diabetes mellitus and altered thyroid function has long been recognized. Both hypothyroidism and hyperthyroidism are reported to be associated with diabetes in humans. Alterations in the concentrations of thyroid hormones can influence glucose homeostasis as well as growth. However, in the earlier studies despite retarded growth of animals, serum levels of T$\text{3}$ and T$\text{4}$ were not determined. In the present study, it was observed that serum T$\text{3}$ and T$\text{4}$ levels of diabetic animals were not significantly different from those of the non-diabetic animals which is in accordance with the earlier observation of unaltered body weight and food and water consumption. Chronic BMOV treatment did not produce any significant effect on serum T$\text{3}$ and T$\text{4}$ levels of non-diabetic and diabetic animals.

Type II diabetes mellitus is also characterized by hyperlipidaemia. In the present investigation, type II diabetic rats showed elevated cholesterol levels compared to non-diabetic control animals indicating altered lipid metabolism. However, serum triglyceride levels were not significantly different from non-
diabetic control rats. Chronic BMOV treatment significantly reduced both serum cholesterol and triglyceride levels in diabetic animals after 6wk treatment indicating improved lipid metabolism upon chronic BMOV administration. Treatment did not produce any significant effect on serum cholesterol levels of non-diabetic animals but significantly reduced serum triglyceride levels of non-diabetic control rats. It has been reported earlier that normalization of lipid parameters was independent of the attainment of euglycemia.

In conclusion, our data suggest that BMOV may be useful adjunct therapy in type II diabetes mellitus which is a cluster of disorders such as hyperinsulinemia, hyperlipidemia, hyperglycemia and insulin resistance, through enhancing effects of insulin and improving glucose and lipid homeostasis.

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