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Effect of sodium molybdate on carbohydrate metabolizing enzymes in alloxan-induced diabetic rats

R. Saraswathi Panneerselvam*, Swaminathan Govindaswamy

Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai - 600 025, India

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Abstract

We evaluated the effect of sodium molybdate on carbohydrate metabolizing enzymes and mitochondrial enzymes in diabetic rats. Diabetic rats showed a significant reduction in the activities of glucose metabolising enzymes like hexokinase, glucose-6-phosphate dehydrogenase, glycogen synthase and in the level of glycogen. An elevation in the activities of aldolase, glucose-6-phosphatase, fructose 1,6- bisphosphatase, glycogen phosphorylase and in the level of blood glucose were also observed in diabetic rats when compared to control rats. The activities of mitochondrial enzymes isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and cytochrome-C-oxidase were also significantly lowered in diabetic rats. Molybdate administration to diabetic rats reversed the above changes in a significant manner. From our observations, we conclude that administration of sodium molybdate regulated the blood sugar levels in alloxan-induced diabetic rats. Sodium molybdate therapy not only maintained the blood glucose homeostasis but also altered the activities of carbohydrate metabolising enzymes. Molybdate therapy also considerably improved the activities of mitochondrial enzymes, thereby suggesting its role in mitochondrial energy production.

Keywords: Diabetes; Sodium molybdate; Carbohydrate metabolizing enzymes

1. Introduction

Diabetes mellitus results from the destruction of the insulin-producing beta cells of the pancreas, a disease development process that can last several years before the clinical onset of the disease [1]. Mitochondria are the most important intracellular source and target of reactive oxygen species. Mitochondrial dysfunction due to enhanced free radical production has been demonstrated in various pathological conditions including diabetes mellitus [2]. Environmental factors such as poor nutrition (high fat diet) or lower oxygen consumption i.e. (VO_2max) or both also seem to have a major effect on the development of peripheral insulin resistance [3]. Diabetes mellitus is associated with a reduced capacity of the β-cells to release sufficient insulin to cover demand, whether the cells are destroyed as in insulin dependent diabetes mellitus (IDDM) or intact as in non-insulin dependent diabetes mellitus (NIDDM). Elevation in the level of non-esterified fatty acids also stimulates gluconeogenesis in the liver [4]. During IDDM, decrease in the insulin : glucagon ratio leads to an increase in both glycogenolysis and gluconeogenesis [5].

The normal beta cell, highly dependent on mitochondrial energy - is the only cell, which increases its function (energy production) during hyperglycemia. During diabetic condition, the activity of the enzyme glucokinase is found to be decreased due to defective insulin release. This inturn affects phosphorylation, the first step in glycolysis which is glucokinase dependent [6]. Thus, glucokinase mutations can directly impair glucose sensing, while mitochondrial DNA mutations can indirectly impair glucose sensing by reducing intracellular concentrations of ATP [7]. Oxidation of glucose-derived acetyl residues increases in a time related and concentration dependent manner when islet or purified β-cells are exposed to a rise in hexose concentration. It was proposed that the increased oxidation of glucose derived acetyl residues is attributed to Ca^{2+} dependent activation of NAD-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase [8].

Recently trace elements like vanadium and tungsten have

* Corresponding author. Tel.: +91-44-18452.
E-mail address: panneerselvam@eth.net (R. Saraswathi).

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been shown to exhibit insulin like properties [9]. Evidence has also been presented to show that molybdenum could affect glucose metabolism *in vitro* [10]. Hence, an earnest attempt has been made in the present study to evaluate the effects of molybdate in correcting the diabetes-associated alterations in carbohydrate metabolizing and mitochondrial enzymes.

2. Materials and methods

Male albino rats weighing between 160–180g were procured from the Veterinary College, Tamilnadu Veterinary University, Chennai, India. The animals were divided into four groups namely: Group I: control, Group II: normal rats orally administered with sodium molybdate (100mg/kg body weight/day) for 30 days, Group III: alloxan-induced animals (140mg/kg body weight intraperitoneally) were considered as diabetic when the fasting blood glucose level showed above 200mg/dl. The blood glucose levels were checked periodically for a month to confirm the presence of uncontrolled hyperglycemic condition and Group IV: diabetic rats treated with sodium molybdate (100mg/kg body weight/day) for 30 days.

On completion of 30 days of molybdate treatment, the animals were sacrificed by cervical decapitation. Blood was collected and used for the estimation of glucose [11]. Liver and kidney tissues were excised immediately and immersed in ice-cold physiological saline. The whole liver tissue was homogenized in 0.1M Tris-HCl buffer (pH 7.4). The particle free homogenate was used for the following analysis: protein was estimated by the method of Lowry et al., (1951) [12] using bovine serum albumin as the standard. Hexokinase activity was measured with respect to the amount of glucose utilized after the addition of ATP [13]. Aldolase activity was assayed according to the method of King (1965) [14] with fructose 1,6-bisphosphate as substrate and dinitrophenyl hydrazine as coloring reagent. Glucose-6-phosphate dehydrogenase activity was measured using 2,6 dichlorophenol indophenol dye according to the method of Ellis and Kirkman (1961) [15]. The activities of glucose-6-phosphatase [16] and fructose1,6-bisphosphatase [17] were assayed with respect to the amount of inorganic phosphorus liberated after the addition of their respective substrates glucose-6-phophate and fructose 1,6-bisphosphate. Glycogen was hydrolyzed by alkali digestion and resulting glucose was estimated according to the method of Morales et al., (1973) [18].

The activity of glycogen synthase was estimated by coupling it with pyruvate kinase activity. It was measured by the amount of uridine diphosphate (UDP) formed from UDP-glucose in the presence of glycogen and glucose-6-phophate. Pyruvate kinase catalyzes the transfer of phosphate from phosphoenolpyruvate to UDP and the pyruvate liberated was estimated colorimetrically [19] The property of synthesizing glycogen from glucose-1-phosphate liberating inorganic phosphate is made use in the assay of glycogen phosphorylase activity [20].

Mitochondria were isolated from fresh [liver and kidney] tissues [21]. The purity of the mitochondria was assessed by estimating the activity of succinate dehydrogenase. This was used for the following analysis: The activity of isocitrate dehydrogenase was assayed by the method of King (1965) [22]. α-Ketoglutarate activity was assayed according to the calorimetric determination of ferrocyanide produced by the decarboxylation of α-ketoglutarate with ferricyanide as electron acceptor [23], and Succinate dehydrogenase activity was assayed by the method of Slater and Bonner (1952) [24], in which the rate of reduction of potassium ferricyanide was measured in the presence of potassium cyanide. The activity of malate dehydrogenase was estimated by the method of Mehler et al. (1948) [25]. The method of Minakami et al. (1962) [26] was followed for the determination of reduced nicotinamide adenine dinucleotide (NADH) - dehydrogenase activity. The activity of cytochrome-C-oxidase was assayed by the method of Warton and Tzagoloff (1967) [27]. All the readings were recorded using UVIKON 810 KONTRON spectrophotometer.

3. Statistical analysis

Values are expressed as mean ± SD for six rats in each group and significance of the differences between mean values were determined by one-way analysis of variance (ANOVA) coupled with Student-Newman-Kuel multiple comparison test. Values of $p < 0.05$ were considered to be significant.

Statistical significance of differences between the control (Group III) and diabetic rats (Group IV) was determined by Student's *t*-test. The levels of significance were evaluated with *p* values.

4. Results

Table 1 shows the activities of carbohydrate metabolizing enzymes and the level of blood glucose (fasting blood) and glycogen in the liver of control, diabetic and molybdate treated diabetic rats. The activities of enzymes hexokinase, G6PDH, glycogen synthase and the level of glycogen were found to be low ($p < 0.001$) whereas the activities of enzymes aldolase, glucose-6-phosphatase, fructose 1,6-bisphosphatase and glycogen phosphorylase were found to be significantly increased ($p < 0.001$) in diabetic rats (Group III) when compared to respective control group (Group I). Molybdate administration to diabetic rats (Group IV) reversed the above changes in a significant manner when compared to diabetic rats (Group III). In normal rats (Group II), molybdate administration for 30 days showed no significant alterations.

Table 2 represents the activities of liver mitochondrial

Table 1
Level of blood glucose and activities of some key enzymes in glucose metabolism in the liver of control, diabetic and molybdate treated diabetic rats

Parameters	Group I (Control)	Group II (Control + Molybdate)	Group III (Diabetic)	Group IV (Diabetic + Molybdate)
Blood glucose (mg/dl)	89.4 ± 4.5	84.7 ± 5.1	247.5[a] ± 19.5***	130.6[b] ± 12.5***
Glycogen	53.2 ± 3.5	52.1 ± 48.3	20.3[a] ± 4.8***	34.3[b] ± 34.***
Hexokinase	263 ± 18.1	272 ± 19.2	116[a] ± 11.0***	209[b] ± 17.3***
Aldolase	168 ± 15.2	162 ± 14.2	275[a] ± 24.3***	205[b] ± 19.2***
Glucose-6-phosphatase	1028 ± 94	970 ± 62	1930[a] ± 143***	1321[b] ± 112***
G6PDH	580 ± 42	568 ± 40	304[a] ± 29***	454[b] ± 26***
Fructose 1,6-bisphosphatase	496 ± 34	454 ± 30	780[a] ± 57***	574[b] ± 43***
Glycogen synthase	840.3 ± 64.1	874.2 ± 73.5	540.2[a] ± 49.0**	748.2[b] ± 50.3***
Glycogen phosphorylase	640.1 ± 45.0	621.3 ± 40.1	870.4[a] ± 58.3***	670.1[b] ± 50.3***

Values are expressed as mean ± SD for six rats in each group.

(Hexokinase - nmoles of glucose-6-phosphate formed/h/mg protein; Aldolase - μmoles of glyceraldehyde formed/h/mg protein; Lactate dehydrogenase- μmoles of pyruvate formed/h/mg protein; Glucose-6-phosphatase - μmoles of Pi liberated/h mg protein; G6PDH - units/min/mg protein; Fructose 1,6-bisphosphatase - μmoles of Pi liberated/h/mg protein; Glycogen - mg of glucose/g wet tissue; Glycogen synthase - μmoles of UDP formed/h/mg protein and Glycogen phosphorylase - μmoles of Pi liberated/h/mg protein).

[a]Group I compared with Group III; [b]Group III compared with Group IV.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

enzymes like isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and cytochrome C oxidase in alloxan induced diabetic rats and molybdate treated diabetic rats. A highly significant decline ($p < 0.001$) in the activities of mitochondrial enzymes were observed in diabetic rats (Group III). Diabetic rats treated with molybdate (Group IV) showed increase in the activities of mitochondrial enzymes viz. isocitrate dehydrogenase (21%), α-ketoglutarate dehydrogenase (35%), succinate dehydrogenase (32%), malate dehydrogenase (31%), NADH-dehydrogenase (49%) and cytochrome C oxidase (63%). Normal rats treated with molybdate (Group II) showed no significant alterations.

Table 3 depicts the activities of mitochondrial enzymes in kidney viz. isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and cytochrome C oxidase in alloxan induced diabetic rats before and after molybdate treatment. The activities of kidney mitochondrial enzymes were markedly lowered in diabetic rats (Group III) when compared with control rats (Group I). 30 days of molybdate therapy significantly enhanced ($p < 0.001$) the activities of these enzymes in Group IV animals when compared to Group III diabetic rats.

5. Discussion

The activities of insulin dependent enzymes like aldolase, hexokinase, glycogen synthase and glucose-6-phosphate dehydrogenase were found to be lowered in the diabetic tissues whereas following molybdate treatment the activities of these enzymes increased in a significant manner. The activities of enzymes catalyzing insulin independent pathways are grossly increased in the untreated diabetic and are reversed during the administration of the drug. The enzymes of the group, which have been studied, are

Table 2
Activities of liver mitochondrial enzymes in control, diabetic and molybdate treated diabetic rats

Parameters	Group I (Control)	Group II (Control + Molybdate)	Group III (Diabetic)	Group IV (Diabetic + Molybdate)
Isocitrate dehydrogenase	786.2 ± 66.40	790.0 ± 69.50	574.2[a] ± 58.30**	694.0[b] ± 64.80**
α-ketoglutarate dehydrogenase	168.1 ± 16.30	172.3 ± 17.40	104.3[a] ± 10.20***	141.0[b] ± 13.60***
Succinate dehydrogenase	23.4 ± 2.10	24.1 ± 2.30	14.6[a] ± 1.10***	19.3[b] ± 1.90***
Malate dehydrogenase	372.0 ± 41.20	369.1 ± 39.60	253.2[a] ± 33.20***	332.5[b] ± 36.70**
NADH-dehydrogenase	28.2 ± 2.80	27.8 ± 2.60	16.3[a] ± 1.20***	24.3[b] ± 2.40***
Cytochrome-C-oxidase	6.83 ± 0.62	6.21 ± 0.58	3.13[a] ± 0.46***	5.12[b] ± 0.52***

Values are expressed as mean ± SD for six rats in each group.

(isocitrate dehydrogenase - nmoles of α-ketoglutarate liberated/h/mg protein; α-ketoglutarate dehydrogenase - μmoles of potassium ferrocyanide liberated/h/mg protein; succinate dehydrogenase - μmoles of succinate oxidized/min/mg protein; malate dehydrogenase - μmoles of NADH oxidized/min/mg protein; NADH-dehydrogenase - μmoles of NADH oxidized/min/mg protein and cytochrome-C-oxidase - O.D. × 10-2min/mg protein).

[a]Group I compared with Group III; [b]Group III compared with Group IV.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 3
Activities of kidney mitochondrial enzymes in control, diabetic and molybdate treated diabetic rats

Parameters	Group I (Control)	Group II (Control + Molybdate)	Group III (Diabetic)	Group IV (Diabetic + Molybdate)
Isocitrate dehydrogenase	686.01 ± 63.40	693.05 ± 64.70	512.03[a] ± 56.30**	603[b] ± 62.10*
α-ketoglutarate dehydrogenase	56.30 ± 5.80	57.10 ± 6.10	34.30[a] ± 3.40***	47.40[b] ± 4.70***
Succinate dehydrogenase	12.40 ± 0.84	12.10 ± 0.79	6.90[a] ± 0.64***	9.40[b] ± 0.87***
Malate dehydrogenase	206.30 ± 23.80	208.40 ± 24.10	114.03[a] ± 15.30**	181.07[b] ± 16.80***
NADH-dehydrogenase	19.30 ± 1.60	18.90 ± 1.70	12.40[a] ± 0.91***	16.30[b] ± 1.30***
Cytochrome-C-oxidase	6.33 ± 0.64	6.41 ± 0.65	3.42[a] ± 0.37***	5.13[b] ± 0.47***

Values are expressed as mean ± SD for six rats in each group.

(isocitrate dehydrogenase - nmoles of α-ketoglutarate liberated/h/mg protein; α-ketoglutarate dehydrogenase - μmoles of potassium ferrocyanide liberated/h/mg protein; succinate dehydrogenase - μmoles of succinate oxidized/min/mg protein; malate dehydrogenase - μmoles of NADH oxidized/min/mg protein; NADH-dehydrogenase - μmoles of NADH oxidized/min/mg protein and cytochrome-C-oxidase - O.D. × 10-2min/mg protein).

[a]Group I compared with Group III; [b]Group III compared with Group IV.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

glycogen phosphorylase, gluconeogenic enzymes like glucose-6-phosphatase and fructose 1,6-bisphosphatase.

The activity of glucose-6-phosphate dehydrogenase has been found to be decreased in the present study. The decrease in the activity of this enzyme in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and thereby the production of reducing equivalents such as NADH and NADPH. Insulin is reported to increase the activity of glucose-6-phosphate dehydrogenase in a dose dependent manner [28]. In our studies, administration of molybdate increased the activity of glucose-6-phosphate dehydrogenase considerably. This may be attributed to the insulin like effect of molybdate. Insulin stimulates the oxidation of glucose via the hexose monophosphate shunt, predominantly in the fat laden cells [29]. A number of investigators have suggested that glucose-6-phosphate dehydrogenase increases the supply of NADPH. Studies regarding therapy with insulin have concluded that the impact of insulin in the liver is to increase glycolysis and to decrease gluconeogenesis, ie., "predominance of glycolysis over glyconeogenesis" [30]. Further insulin integrates hepatic carbohydrate metabolism by increasing the biosynthesis of enzymes of glycolysis, glycogenesis, pentose oxidative pathway and by inhibiting gluconeogenesis [31]. These results indicate that the increase in the activity of glucose-6-phosphate dehydrogenase may be owed to the sequence of changes following molybdate administration which possesses insulin mimicrying action.

Diabetes mellitus is associated with a marked decrease in the level of liver glycogen. The reduced glycogen store has been attributed to the reduction in the activity of glycogen synthase and an increase in the activity of glycogen phosphorylase. This results in an increased blood glucose level typical for diabetes. The activity of glycogen synthase in the liver is found to be decreased in the diabetic animals. This may be the result of low levels of circulating insulin, which is an inducer of glycogen synthase. Administration of molybdate restored back the activity of the enzyme to normal on 30 days of treatment. Phosphorylase activity in diabetic rat tissues shows an increase in the level of the active 'a' form. This is parallel to the finding of Witters and Auruch [32] in which insulin administration increased the activity of glycogen synthase and decreased the activity of glycogen phosphorylase. Under conditions of insulin deficiency, the activity of this enzyme is found to be elevated, as observed in our studies. Molybdate administration favored a decrease in phosphorylase, an effect closely resembling insulin.

In the present study, the observed decrease in the activities of mitochondrial enzymes in liver and kidney of the diabetic rats were significantly enhanced upon molybdate therapy (Table 2, 3). In insulin dependent diabetes mellitus (IDDM) various agents like interleukin-1 beta, interferon gamma, tumor necrosis factor alpha, alloxan and streptozotocin - could operate by forming free radicals that could attack the mitochondrial genome [33]. The increased production of free radicals in mitochondria may damage β-cells, which is known to be very sensitive to free radicals [34]. Also, a decrease in oxygen consumption and respiratory ratio were observed in the mitochondria of diabetic rats [35]. A similar decrease in the activities of citric acid cycle enzymes were also observed by Sener et al., (1990) [36]. Furthermore, lowering in the activities of pyruvate dehydrogenase and malate dehydrogenase and increase in NAD^+/NADH ratio were reported by Obrosova et al. (1999) in alloxan-induced diabetic rats [37]. It has been suggested that the diabetogenicity of streptozotocin is dependent on the inhibition of the activities of citric acid cycle enzymes like isocitrate and α-ketoglutarate dehydrogenase [38].

In diabetes mellitus, abnormalities of mitochondrial enzymes may impair the metabolism of glucose. As the rate of glucose oxidation normalizes insulin secretion and subsequent release of β-cells, a defective insulin response to glucose stimulation may be due to respiratory chain deficiency in the pancreas of IDDM. This supposes that random partitioning of mitochondria during development might have resulted in the accumulation of mutated mitochondrial DNA-containing fragments in particular tissues including

pancreas. The rearrangements produce potentially antigenic chimeric proteins. The reduction in the functioning of mitochondrial enzymes may lead to a defect in the mitochondrial energy production which would impair protein synthesis and energy production in β-cell [39]. Restoration of the activities of mitochondrial enzymes on molybdate therapy suggests that it may be beneficial in enhancing protein synthesis and energy production in β-cells.

Vanadate has been found to induce a cytosolic protein tyrosine kinase in rat adipocytes and the activity of this 53KDa cytosolic protein tyrosine kinase (cytPTK) has been correlated with that of insulin receptor tyrosine kinase and hence to the insulin mimetic activity of vanadate [40]. The cytPTK is activated by auto phosphorylation and deactivated by protein phosphotyrosine phosphatase (PTPases). It has been postulated that vanadate inhibits PTPases and thus retains the activity of cytPTK [40]. Recently, an increase in the activity of cytPTK has been observed for molybdate also [10]. This suggests that both vanadate and molybdate share common intracellular pathways to elicit their insulin like effects.

Therapy with insulin for 2 weeks helped to restore the activities of mitochondrial citric acid cycles enzymes, whereas continuing the therapy for 4 weeks fully reversed the activities of these enzymes [36]. It was further observed that ATP production was normalized after insulin therapy [40]. However, direct insulin like action as observed *in vitro* [41] is also likely to play a determinant role in the *in vivo* effects of molybdate noted in the present study. Another possible mechanism for the molybdate mediated anti-diabetic role is the lowering in the levels of non-esterified fatty acids, with subsequent attenuation of the glucose fatty acid cycle which may improve glucose utilization in peripheral tissues and thus contribute to the beneficial effect of molybdate [10].

The present data on the effect of molybdate on alloxan-induced diabetic rats indicates that the impairment in the activities of glucose-metabolizing enzymes, in cytosol and mitochondria have been corrected by the insulin like action of molybdate and reveals the possible utility of molybdate in orthomolecular medicine to treat diabetes mellitus.

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