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Chronic cobalt treatment decreases hyperglycemia in streptozotocin-diabetic rats

Harish Vasudevan & John H. McNeill*
*Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver, BC, V6T 1Z3, Canada *Author for correspondence (e-mail: jmcneill@interchange.ubc.ca phone: +1-604-822-9373 fax: +1-604-822-8001)*

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Abstract

Diabetes is a metabolic disorder characterized by elevated blood glucose levels. Although conventional treatments such as insulin and other drugs reduce blood glucose, there is still a therapeutic need for effective orally administered drugs. Trace elements like vanadium and tungstate have been successfully demonstrated to reduce blood glucose in experimental diabetes with minimal chronic complications. We investigated the anti-hyperglycemic effects of cobalt in streptozotocin-diabetic rats. Normal and diabetic rats were provided with drinking water containing 3.5 mM cobalt chloride for three weeks followed by 4 mM for four weeks. Body weights and fluid consumption were monitored on a daily basis, while food intake was recorded twice every week. Prior to termination, an oral glucose tolerance test was performed on the animals. Diabetic rats lost significant body weight (357 ± 2 gm) compared to controls (482 ± 3 gm). Body weight was further reduced by cobalt treatment (290 ± 2 gm). Although it was difficult to establish a dosing regimen without weight loss, food and fluid consumption in cobalt-treated diabetic rats improved significantly compared to untreated diabetics. Plasma glucose levels were significantly reduced with reference to diabetic controls (29.3 ± 0.9 mM) by the fourth week to a lower but still hyperglycemic level (13.6 ± 3.4 mM). Cobalt-treated diabetic rats demonstrated an enhanced ability to clear a glucose load compared to untreated diabetics. Cobalt treatment neither affected the feeding and drinking patterns nor plasma glucose in normoglycemic animals although body weights decreased compared to untreated controls. We conclude that chronic cobalt treatment decreases plasma glucose levels in STZ-diabetic rats and improves tolerance to glucose.

Introduction

Type-1 diabetes (T1D) is a metabolic disorder characterized by elevated blood glucose levels and hypoinsulinemia as well as polydipsia, polyphagia and polyuria and a reduction in body weight. Most of the conventional treatments such as insulin act by reducing blood glucose, thus normalizing other secondary effects. However due to limitations in insulin therapy such as route of administration and possible hypoglycemia, there is still a therapeutic need for orally effective drugs.

Trace elements have been shown to have anti-hyperglycemic activity in animal models of diabetes. Vanadium is one such element which has been shown to reduce blood glucose without appreciable side effects (Pederson *et al.* 1989). Recently tungstate has been reported to be an effective antidiabetic agent in experimental diabetes with minimal chronic complications (Barbera *et al.* 1994; Nagareddy *et al.* 2005).

Cobalt, a trace element in the body, is a transition element belonging to group VIIIB in the periodic table. It has been previously reported that

cobalt possesses glucose-lowering properties (Ybarra *et al*. 1997). Its action has been mainly ascribed to increased GLUT1 expression (Ybarra *et al*. 1997) and inhibition of gluconeogenesis (Saker *et al*. 1998) in STZ-diabetic rats. Cobalt has been used either as cobalt chloride or as a dipicolinate complex (Yang *et al*. 2002). To date, results obtained are from relatively acute studies (10–16 days) with relatively low doses. Further the glucose levels, although significantly reduced, were still in the hyperglycemic range. Cobalt has been reported to have toxic effects when given in higher concentrations (Clyne *et al*. 2001), thus making its effects controversial. In this study, we report the effects of cobalt on the markers of diabetes in rats injected with streptozotocin (STZ), a pancreatic β-cell toxin. Our studies demonstrate that chronic treatment using cobalt decreases plasma glucose in STZ-diabetic rats.

Materials and methods

Twenty-six male Wistar rats weighing 260–300 gm were obtained from Charles River, Montreal. They were cared for in accordance with the guidelines laid out by the Canadian Council on Animal Care. The animals were acclimatized to the viviarium; following which they were randomly divided into 4 groups of control (C, $n = 6$), diabetic (D, $n = 7$), control treated (CT, $n = 6$) and diabetic-treated (DT, $n = 7$). Half of the animals were made diabetic with a single intravenous injection of streptozotocin (STZ – 60 mg/kg in isotonic saline) into the tail vein. Blood glucose was measured 72 hours after injection of STZ for confirmation of hyperglycemia. One week after the development of diabetes, 3.5 mM cobalt chloride (Sigma Chemical Company, St. Louis, MO) was introduced in the drinking water of treated groups (CT and DT), while the control groups received normal tap water. All animals had *ad libitum* access to laboratory rat chow. Rats were monitored on a daily basis for changes in body weight, fluid intake and physical appearance. Changes in food consumption were assessed twice a week and an average was obtained. Blood was collected every week from the tail and centrifuged at 10,000 g for 25 min at 4 °C. The plasma thus obtained was used for determining plasma glucose and insulin, the latter of which was done every second week. At the end of 3 weeks, the concentration was increased to 4 mM cobalt. After 6 weeks of cobalt treatment, an oral glucose tolerance test (OGTT) was performed to assess changes in glucose metabolizing capacities and corresponding changes in insulin sensitivity. Rats were orally gavaged with 1 gm/kg dose of a 40% glucose solution. Blood was withdrawn from the tail at 0, 10, 20, 30 and 60 min for measurement of plasma glucose and insulin. The plasma was separated by centrifugation and stored at −80 °C until analyzed. All animals were treated for 1 more week following the OGTT and euthanized using a single intraperitoneal injection of pentobarbital (Somnotol^{TM}) 60 mg/kg.

Prior to this study a pilot experiment to standardize the dose of $CoCl_2$ was conducted using 6 STZ-diabetic animals in which the same protocol as described above was followed.

Biochemical analysis

Plasma glucose was determined using a Beckman Glucose Analyzer II (Beckmann, Fullerton, Ca). Plasma insulin was measured using radioimmunoassay (RIA) kits from Linco Research Inc. (Ann Arbor, MI)).

Statistical analysis

All values unless specified were expressed as means ± SEM. Differences among groups were compared by one-way ANOVA followed by Newman–Keuls multiple comparison test. Mean differences were considered significant at $P < 0.05$

Results

General characteristics and physical status

Administration of STZ rendered the rats diabetic, characterized by hyperglycemia, hypoinsulinemia and decreased body weight gain along with increased food and fluid intake when compared to age-matched controls. Cobalt treatment did not result in any overt signs of toxicity or mortality in either control or diabetic rats. However, diabetic rats treated with cobalt (DT) showed a significant reduction in body weight compared to untreated controls (C). Cobalt treatment also reduced the body weight gain in control rats (CT; Figure 1).

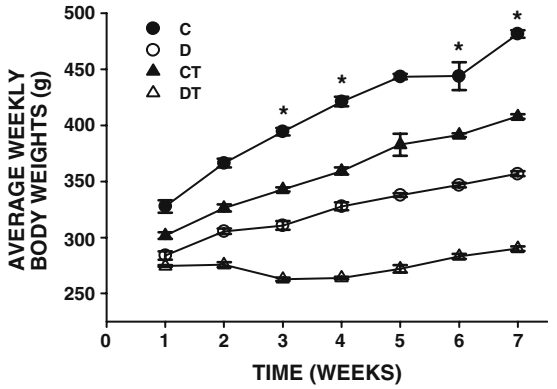

Figure 1. Average weekly body weights. Body weights were measured daily for 7 weeks. Diabetic animals (D) had a lesser weight gain compared to untreated controls, while the diabetic treated (DT) rats gained lesser weight compared to basal values. Cobalt also reduced the weight gain in control treated group (CT) Values are means ± SEM. *$P < 0.05$ C vs. D, CT & DT; +$P < 0.05$ CT vs. D & DT; α $P < 0.05$ D vs. DT respectively.

The decrease in body weight gain compared to untreated controls was observed in the diabetic untreated and in both the treated groups throughout the course of the study.

Pronounced hyperglycemia was achieved in rats at the end of 72 hours following STZ injection (D: 23.7 ± 6.8 vs. C: 8.1 ± 6.1 mM). Although treatment with 3.5 mM cobalt for 3 weeks decreased plasma glucose levels in diabetic rats, the rats were still hyperglycemic compared to controls (Figure 2). The increase in $CoCl_2$ concentration from 3.5 mM to 4 mM resulted in a further drop in the glucose levels (13.6 ± 3.6 mM) at the end of 4 weeks. Furthermore, 4 rats out of 7 had glucose levels below 7.6 mM. At weeks 5 and 6, the glucose levels were in the DT rats were 17.3 ± 3.5 and 18 ± 2.7 mM respectively (Figure 2).

Diabetic rats consumed more fluid and food than either control or control treated rats throughout the treatment period. Although the diabetic treated rats consumed more fluid in weeks 1 and 2, consumption was reduced, beginning with week 3, to values similar to control groups (Figure 4). Cobalt treatment normalized the food intake in diabetic rats (DT) from week 1 as compared to untreated diabetics (Figure 3). The amount of food consumed by DT rats was similar to C and CT.

Oral glucose tolerance test

Following a 16 hour fast, diabetic rats had higher levels of plasma glucose compared to other groups (Figure 5a) and higher plasma glucose levels at every time point following the administration of glucose. Diabetic treated rats had lower fasting glucose, which increased post-challenge. However the glucose levels in DT rats were significantly lower than in the D rats. Insulin levels were similar in D and DT animals throughout the study (Figure 5b; See insert). Cobalt treatment did not affect glucose tolerance in the control animals.

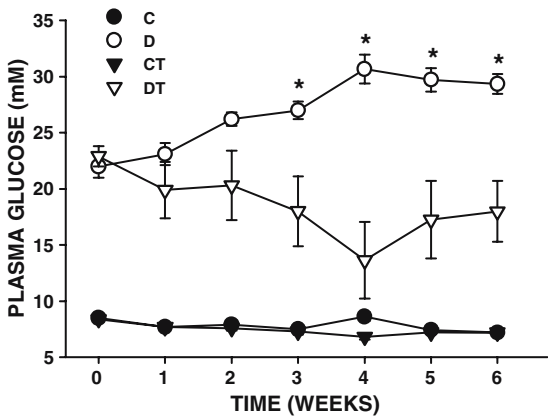

Figure 2. Changes in plasma glucose in C, D, CT and DT rats. Values are means ± SEM. Data are from the start of treatment. Diabetic rats had higher glucose value compared to controls. Cobalt reduced plasma glucose after 3 weeks of treatment, which was observed throughout the study. *$P < 0.05$ D vs. C, DT & CT; +$P < 0.05$ DT vs. C & CT respectively.

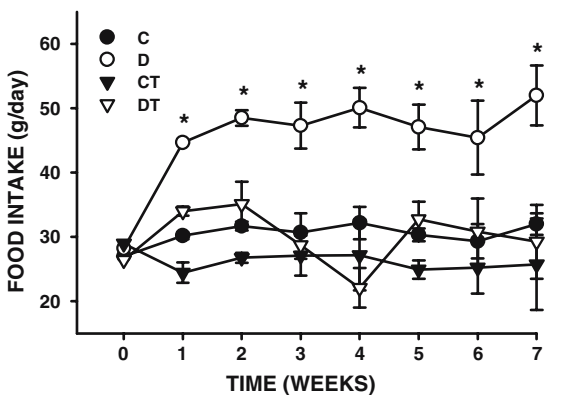

Figure 3. Changes in food intake. Food intake was measured twice every week. Diabetic untreated animals consumed higher mounts of food compared to controls. Cobalt treatment restored food intake to control levels. Cobalt did not affect the food intake in the non-diabetic controls. Values are means ± SEM. *$P < 0.05$ vs. C, C & DT.

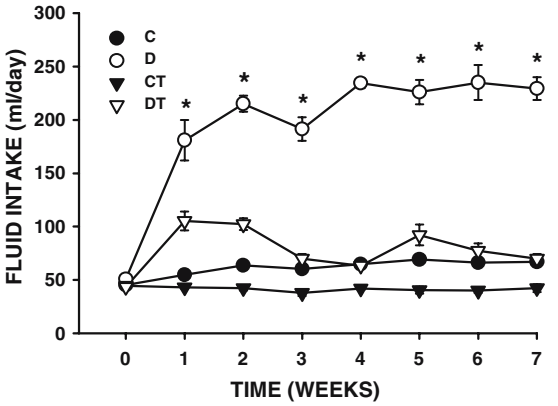

Figure 4. Changes in fluid consumption. The induction of diabetes resulted in increased consumption of water as compared to control. Cobalt normalized the fluid consumption in diabetic rats. Cobalt treatment did not alter the fluid consumption in control rats. Values are means ± SEM. *$P < 0.05$ vs. C, C & DT.

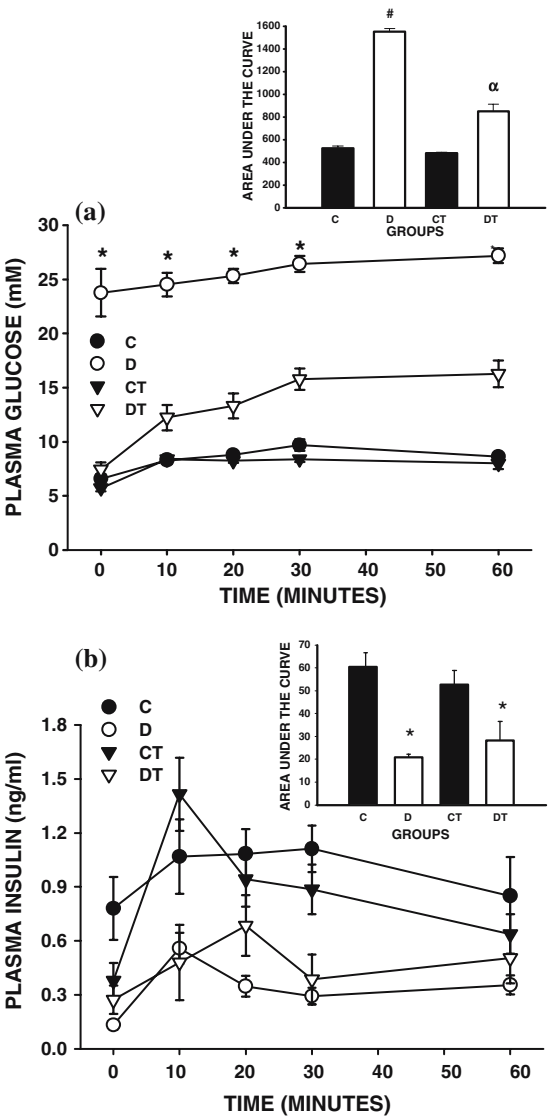

Figure 5. (a) Changes in plasma glucose following OGTT. Glucose was measured at 0, 10, 20, 30 and 60 min after glucose administration. Cobalt decreased glucose in diabetic animals (see insert on AUC). Cobalt did not affect glucose clearance was in control animals Values are means ± SEM. *$P < 0.05$ D vs. C, CT & DT; # $P < 0.05$ DT vs. C & CT respectively. (b) Changes in plasma insulin following OGTT. Insulin was measured at 0, 10, 20, 30 and 60 min after glucose administration. Cobalt had no effect on the AUC of insulin levels (see insert). Values are means ± SEM. *$P < 0.05$ vs. C & CT.

Discussion

In the present study, we have demonstrated that chronic administration of the trace element cobalt decreases plasma glucose in STZ-diabetic rats. The effect was achieved without alterations in plasma insulin levels. Our results are similar to previous reports from our laboratory (Cam *et al*. 1993; McNeill *et al*. 1994; Nagareddy *et al*. 2005), in which we showed that treatment with vanadium or tungstate reduced hyperglycemic glucose levels to lower levels without affecting the amount of insulin secreted. This suggests an insulinomimetic or insulin-enhancing effect of cobalt. Thus, cobalt may mimic insulin in its glucose-regulatory actions or it may merely enhance the effects of the insulin present in the rat. In other words, the amount of insulin available to STZ-diabetic rats is capable of metabolizing higher concentrations of glucose in the presence of cobalt. In the present study, we found no statistical differences between the insulin levels of diabetic and diabetic-treated rats following the overnight fast. In addition, plasma insulin levels did not vary between the responders and the non-responders in the diabetic-treated rats (DT).

In our studies, treatment with cobalt significantly reduced the gain in body weight of both control (CT) and diabetic (DT) rats (Figure 1). This is in agreement with previous reports where treatment with 2 mM cobalt for 2 weeks resulted in a drop in the weight gain (Ybarra *et al*. 1997). In agreement with Ybarra *et al*., we also observed that cobalt decreased the food and water consumption in the diabetic rats (Figures 3, 4). Treatment with 2 mM cobalt for 2 weeks decreased plasma glucose levels (Saker *et al*. 1998; Ybarra *et al*. 1997). In comparison with basal

values (not shown), the consumption of water was significantly lower in the diabetic treated rats from the first week of treatment as compared to untreated diabetics. Although the consumption of water in the diabetic treated rats was higher than controls in the initial 2 weeks, it subsequently was reduced to values similar to controls. The decrease in fluid intake in DT rats is likely due to the decrease in plasma glucose, which would reduce the fluid loss in the urine.

In our experiments, cobalt decreased plasma glucose by the second week, was pronounced in the third and fourth weeks and finally stabilized in the last 2 weeks of treatment. Treatment with 3.5 mM and subsequently 4 mM $CoCl_2$ resulted in a greater degree of reduction in the plasma glucose levels of STZ-diabetic rats (18 mM glucose at 3.5 mM vs. 13.6 mM glucose at 4 mM $CoCl_2$). Interestingly, at the end of 4 weeks, plasma glucose levels were reduced to less than 7.6 mM in more than 50% of the rats and was partially reversed at the end of the treatment. This may indicate that rats vary in their responses to cobalt treatment.

Previous reports have speculated on the possibility of an increase in sensitivity to insulin subsequent to treatment with cobalt (Eaton 1972; Ybarra *et al.* 1997). We have demonstrated that chronic consumption of cobalt in diabetic rats, while not affecting insulin levels, improves glucose tolerance. However, cobalt did not affect the insulin levels in the control rats. Cobalt has been suggested to modulate specific mediators and/or pathways involved in glucose metabolism. Some of these are increased GLUT-1-mediated glucose transport (Ybarra *et al.* 1997) and decreased hepatic gluconeogenesis (Saker *et al.* 1998). Cobalt has also been shown to normalize hepatic glycogen levels in STZ rats (Nomura *et al.* 2005) although its effects on glucagon are unclear. Furthermore, one of the mechanisms suggested to decrease hyperglycemia is to attenuate the oxidative insult induced by diabetes. Treatment with antioxidants such as n-acetylcysteine has been shown to improve the insulin secretion profile and therefore reduction in hyperglycemia (Kaneto *et al.* 1999). Cobalt has been previously demonstrated to decrease lipid peroxidation in STZ-diabetic rats in various organs such as the liver (Yildirim & Buyukbingol 2002), heart and aorta (Yildirim & Buyukbingol 2003). The beneficial effects of $CoCl_2$ treatment has been demonstrated on the heart-one of the key target organs of secondary complications, wherein low concentrations of cobalt improved cardiac contractility (Endoh *et al.* 2000). However its effects on diabetic cardiomyopathy and other chronic complications need to be investigated. Thus it is possible that the antioxidant action of cobalt may contribute to its glucose lowering effects.

In our pilot studies, diabetic rats responded variably to increasing concentrations of $CoCl_2$ (2, 3.5 and 4 mM). While there was no change in plasma glucose levels over 2 weeks subsequent to treatment with 2 mM $CoCl_2$, increasing the concentration to 3.5 mM and subsequently 4 mM decreased plasma glucose. All concentrations were well tolerated by the rats for a period of 7 weeks. We did not experiment with higher concentrations as it has been previously reported that concentrations of $CoCl_2$ of 6 mM were toxic in rats and resulted in diarrhea and weight loss (Saker *et al.* 1998). Although a few diabetic rats lost weight during the study, the weights were restored subsequent to replacing cobalt in these rats with normal drinking water for 24–48 hours (Data not shown). Further, we did not find any significant change in glucose levels when the treatment duration was extended to 7 weeks.

In summary, chronic treatment with cobalt chloride decreased hyperglycemia in STZ-diabetic rats. We suggest that this effect is brought about by the antioxidant and insulin enhancing actions of cobalt.

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