ORIGINAL ARTICLE

Effect of 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) on hexavalent chromium (Cr (VI)) induced alteration of glucose homeostasis in Wistar rats

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Abstract

Background: 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) is a fat-soluble known antioxidant vitamin to protect cardiovascular health. Hexavalent chromium (Cr (VI)) as a heavy metal has adverse effects on vascular system. *Aim and Objectives*: To evaluate the possible protective effects of $1,25(OH)_2D_3$ supplementation on Cr (VI) induced altered glucose regulation. *Materials and Methods:* Twenty-four adult Wistar male rats were divided into four groups (n=6 in each group) as following: Group-1 (control); Group-2 received $K_2Cr_2O_7$ 5.0 mg/kg body weight intraperitoneally for 10 dosages on every alternate day for 20 days; Group-3 received $1,25(OH)_2D_3$ 12.5µg/kg/d, orally daily till 20 days; and Group-4 received both $K_2Cr_2O_7$ and $1,25(OH)_2D_3$ with dosages as above. At the end of 10^{th} dosage after overnight fasting i.e. (on day 21) blood samples were collected from tail vein of all the rats. Oral Glucose Tolerance Test (OGTT), serum glucose and insulin concentrations were estimated. Insulinogenic index was also calculated. Liver glycogen concentrations were assessed after sacrificing the animals. *Results:* OGTT showed an increase of fasting blood glucose levels in Cr (VI) treated Group-2 rats till at the end of 2.0 hrs. The Cr (VI) treated and simultaneously supplemented with $1,25(OH)_2D_3$ Group-4 rats showed lesser elevation of blood glucose level till 2.0 hrs. A decrease in plasma insulin level and increase in insulinogenic index were also found in Cr (VI) treated Group-2 rats but in case of vitamin D_3 supplemented Group-4 rats, both plasma insulin levels and insulinogenic index were found to be improved remarkably. Liver glycogen concentrations in $K_2Cr_2O_7$ treated rats were also found to be reduced significantly but $1,25(OH)_2D_3$ supplemented Group-4 rats showed improvement in liver glycogen concentrations. *Conclusion:* $1,25(OH)_2D_3$ is found to be beneficial against hexavalent chromium induced alteration of glucose homeostasis.

Keywords: Chromium (VI), Glucose Homeostasis, 1,25-dihydroxy vitamin D_3

Introduction

Chromium is a heavy metal routinely used in alloy industries. Although chromium (III) is an important trace element required for insulin synthesis, Cr (VI) or hexavalent chromium possesses several toxicological symptoms. Industrial uses of chromium are mainly as a hexavalent form. Several studies on humans and animals found that hexavalent chromium has both acute and chronic toxicities, which include even cancer-like diseases [1]. Cr (VI) toxicity depends on the dosages and duration of exposure. Long time exposure with higher dosages of Cr (VI) upregulates apoptosis genes and down regulates antioxidant genes like Superoxide Dismutase (SOD), Glutathione (GSH) etc. [2]. Cr (VI), which is a powerful oxidant that gets converted into Cr (III) generates Reactive Oxygen Species

(ROS) and is capable of damaging intracellular organelles and developing toxicities. Cr (VI) targets multiple physiological systems in the body i.e. hematological and digestive, respiratory system, besides metabolically active organs like liver, kidney and brain, which results in serious toxic manifestations [3]. There are very few reports on Cr (VI) induced hyperglycemia in experimental setup with suggested possibilities of remediation by some phytochemical supplementations due to their antioxidant properties [4]. As Cr (VI) is not stable in the body, it is reduced to relatively nontoxic Cr (III) in the presence of antioxidants [5]. Normally reduction of Cr (VI) is the process of detoxification which may occur inside or outside the cell. The detoxification process of Cr (VI) within the cell is hazardous as it damages the cell organelles and DNA, whereas detoxification outside of the cell by the reduction of Cr (VI) is relatively less toxic [6].

1,25-Dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) is synthesized indigenously in the body and plays an antioxidant defense against oxidative stress in the body. Vitamin D_3 ($1,25,(OH)_2CC$), which is the biologically active form of vitamin D_3 is synthesized in the kidney and is found to be a protective factor as a possible antioxidant against metabolic diseases, including cardiovascular ailments [7]. Some studies showed the protective action of antioxidants against Cr (VI) induced oxidative stress. There are some reports on Cr (VI) and alteration of glucose metabolism due to oxidative stress but the role of antioxidants like $1,25(OH)_2D_3$ against Cr (VI) toxicities has not been fully understood. Hence the present study assessed the protective role of $1,25(OH)_2D_3$ supplementation against Cr (VI) treated alteration of glucose homeostasis.

Material and Methods

Experimental Animals: Twenty-four adult Wistar strain of male rats (*Rattus novergicus*) of 8-10 weeks old, weighing 180-220 g body weight, were procured from the Central Animal House of Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura. Before experimental protocols, rats were acclimatized for 7 days in laboratory conditions (temperature of $22^0C \pm 2^0C$ and 12 hrs of light-dark cycles). Institutional Animal Ethics Committee clearance was taken.

All the experimental animals were pair-fed with normal laboratory stock diet and water *ad libitum*. All the experimental protocols were performed according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

Experimental groups: Animals were divided into four groups with six in each group and given intervention as described in Table 1.

Table 1: Experimental groups and interventions for 21 days

Groups	Number of rats	Intervention
Group-1 (Control)	n=6	Placebo, oral gavage daily
Group-2 ($K_2Cr_2O_7$)	n=6	$K_2Cr_2O_7$ 5.0 mg/kg b.wt., i.p. for 10 dosages on every alternate day [8].
Group-3 ($1,25(OH)_2D_3$)	n=6	$1,25(OH)_2D_3$ 12.5 µg/kg/d, orally daily till 20 days [9].
Group-4 ($K_2Cr_2O_7$ + $1,25(OH)_2D_3$)	n=6	$K_2Cr_2O_7$ 5.0mg/kg b.wt., i.p. for 10 dosages on every alternate day + $1,25(OH)_2D_3$ 12.5 µg/kg/d, orally daily till 20 days.

b.wt: body weight; i.p: intraperitoneally

Glucose homeostasis analysis

Blood glucose estimation: Blood was collected from the tail vein of all the rats and fasting blood glucose levels were measured by using the oxidase-peroxidase method on day 1, day 7, day 14 and day 21 [10].

Oral Glucose Tolerance Test (OGTT): All the rats of four groups were orally fed with glucose (3.5 g/kg b.wt.) on day 21. Just immediately before glucose administration (0.0 hrs), blood samples were collected from the tail vein of all the rats on every 0.5 hrs intervals till 2.0 hrs. Blood glucose level were immediately measured by using a glucometer (Accu-chek active, Roche diagnostics, Germany)

Plasma insulin and insulinogenic index: Collected blood samples from all the four groups were kept in heparinized microtubes and immediately placed in ice bath for 20 minutes and centrifuged for 7-10 minutes at 4000 rpm for plasma separation. Plasma insulin concentrations were measured at 0 and 30 min (0.5 hr) after glucose administration by ELISA kit (ERINS, Thermo Fischer Scientific, Life technologies). The insulinogenic index was also calculated by using the following formula:

Insulinogenic index = (30 min plasma insulin – fasting plasma insulin) / (30 min plasma glucose – fasting plasma glucose) [11].

Liver glycogen estimation: Animals were sacrificed after blood collection, and liver tissue was isolated from each rat. Liver glycogen was estimated by using a glycogen assay kit (Abnova, Taiwan; catalogue number KA0861 [12].

Statistical analysis

All analysis were done by using SPSS 2 version of software. Mean ± SD of each group was done. One-way ANOVA followed by Post Tukey's multiple comparison tests were also done to find out significant differences between groups ($p < 0.05$).

Results

Hexavalent chromium ($K_2Cr_2O_7$) treatment (5.0 mg/kg b.wt., i.p.) in Group-2 progressively increased fasting blood glucose level on D7, D14 and D21 (Figure 1). Hexavalent chromium treated and simultaneously $1,25(OH)_2D_3$ supplemented (12.5 µg/kg/d, orally) rats in Group-4 showed a decrease in fasting blood glucose level after D14 onwards till D21 (Figure 1).

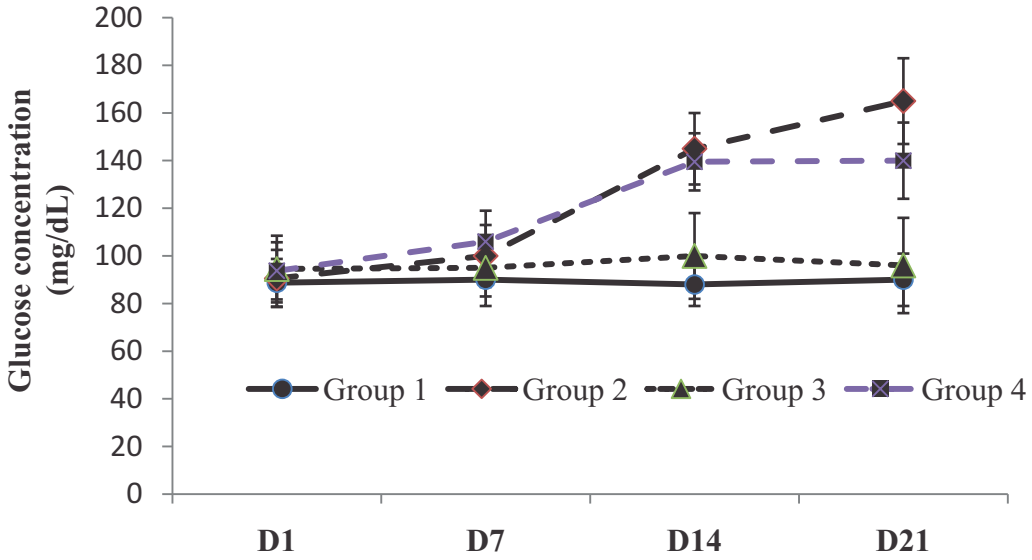

Figure 1: Effect of vitamin D_3 (12.5 µg/kg/d, orally) supplementation on $K_2Cr_2O_7$ (5.0 mg/kg b.wt., i.p. 10 dosages) treated rats on fasting blood glucose concentration (mg/dL) among four groups of rats on day 1 (D1), day 7 (D7), day 14 (D14) and day 21 (D21). Group-1, control; Group-2, $K_2Cr_2O_7$; Group-3, vitamin D3; Group-4, $K_2Cr_2O_7$ + vitamin D_3; n=6 rats in each group.

Table 2 shows a normal OGTT in Group-1 (control) rats i.e. gradual increase of glucose level till 1.0 hr and followed by decrease of blood glucose levels. At 2.0 hr the value became near normal level. OGTT also shows that Group-2 blood glucose levels from 0.0 hr to till 2.0hrs remained higher than baseline value. All the values of Group-2 were also found to be significantly higher than Group-1 from baseline FBS to till 2.0 hr at any given duration during OGTT ($p<0.05$). In Group-4 $1,25(OH)_2D_3$ supplemented $K_2Cr_2O_7$ treated rats showed significant decrease in blood glucose level at every interval till 2.0 hr although it never reached to similar baseline value even at 2.0 hr but as compared to Group-2 ($K_2Cr_2O_7$) all the values of Group-4 at any interval were significantly lower ($p<0.05$).

Table 3 shows fasting plasma glucose, insulin, insulinogenic index and liver glycogen concentrations. Results show an increase of fasting glucose level with significant decrease of plasma insulin level in hexavalent chromium treated rats (Group-2) as compared to controls (Group-1) (($p < 0.05$)). In case of Group-4 ($K_2Cr_2O_7$ + $1,25(OH)_2D_3$), plasma level of glucose was found to be decreased and plasma level of insulin was found to be increased as compared to Group-2 i.e. hexavalent chromium treated rats ($p<0.05$). Insulinogenic index indicates an increased value in Group-2 ($K_2Cr_2O_7$) rats whereas in case of Group-4 ($K_2Cr_2O_7$ + $1,25(OH)_2D_3$) it was found to be decreased. Results from Table-3 are also showing a decreased value of liver glycogen in group-2 hexavalent chromium treated rats as compared to Group-1 (control). The liver glycogen concentration in Group-4 ($K_2Cr_2O_7$ + $1,25(OH)_2D_3$) rats shows a significant increase as compared to Group-2 rats ($p<0.05$) although it remained lower as compared to Group-1 (control).

Table 2: $1,25(OH)_2D_3$ (12.5 µg/kg/d, orally) supplementation on OGTT in sub chronic $K_2Cr_2O_7$ (5.0 mg/kg b.wt., i.p.) treated rats on D21 (at the end of 10 dosage)

Treatment groups	0.0 hr	0.5 hr	1.0 hr	1.5 hr	2.0 hr	*p*
Group 1	$89.00 \pm 10.56^{a, x}$	$110.54 \pm 10.56^{b, x}$	$113.76 \pm 10.65^{b, x}$	$100.54 \pm 10.76^{c, x}$	$84.45 \pm 10.43^{a, x}$	< 0.05
Group 2	$30.54 \pm 14.75^{a, y}$	$156.76 \pm 15.43^{b, y}$	$158.76 \pm 12.45^{b, y}$	$170.56 \pm 12.65^{c, y}$	$170.75 \pm 11.24^{c, y}$	< 0.05
Group 3	$78.96 \pm 6.06^{a, x}$	$113.76 \pm 10.65^{b, x}$	$113.75 \pm 15.75^{b, x}$	$107.76 \pm 16.50^{b, x}$	$95.56 \pm 16.34^{c, x}$	< 0.05
Group 4	$112.75 \pm 10./65^{a, z}$	$125.75 \pm 12.50^{b, z}$	$134.56 \pm 10.87^{c, z}$	$120.76 \pm 10.97^{b, z}$	$119..65 \pm 9.56^{b, z}$	< 0.05
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	

Group-1, control; Group-2, $K_2Cr_2O_7$; Group-3, $1,25(OH)_2D_3$; Group-4, $K_2Cr_2O_7$ + $1,25(OH)_2D_3$. n=6 rats in each group. D21, day 21. In each row, values with different superscripts (a, b, c) are significantly different from each other ($p < 0.05$). Vertical columns indicate variation of blood glucose level among four different groups at different time interval till 2 hrs. In each column, values with different superscripts (x, y, z) are significantly different from each other ($p<0.05$).

Table 3: $1,25(OH)_2D_3$ supplementation (12.5 µg/kg/d, orally) on fasting plasma glucose, insulin and insulinogenic index and liver glycogen in $K_2Cr_2O_7$ treated rats (5.0 mg/kg b.wt., i.p.) D21 (at the end of 10 dosages)

Treatment	Fasting plasma glucose (mg/dL)	Fasting Plasma Insulin (µg/L)	Insulinogenic index	Liver glycogen (mg/g)
Group-1	87.27 ± 3.85^{a}	1.07 ± 0.17^{a}	0.0034 ± 0.00052^{a}	20.56 ± 1.49^{a}
Group-2	137.65 ± 12.29^{b}	0.54 ± 0.09^{b}	0.0058 ± 0.00064^{b}	14.14 ± 1.18^{b}
Group-3	86.91 ± 3.65^{a}	1.11 ± 0.14^{a}	0.0031 ± 0.00029^{a}	20.93 ± 1.55^{a}
Group-4	113.08 ± 3.42^{c}	0.75 ± 0.22^{c}	0.0052 ± 0.00035^{c}	17.53 ± 2.11^{c}

Group-1, control; Group-2, $K_2Cr_2O_7$; Group-3, $1,25(OH)_2D_3$; Group-4, $K_2Cr_2O_7$ + $1,25(OH)_2D_3$. n=6 rats in each group. D21, day 21. Values with different superscripts are significantly different from each other ($p<0.05$)

Discussion

Results from figure 1 indicate a steady elevation of fasting blood glucose level in hexavalent chromium ($K_2Cr_2O_7$) treated rats from day 7 onwards, and it remained in elevated status even at the end of the experiment (D21). It indicates that hexavalent chromium is a hyperglycemic metal, but simultaneous supplementation of $1,25(OH)_2D_3$ can control hyperglycemia from D14 onwards. The results also reflect heavy metals like $K_2Cr_2O_7$-induced duration-dependent steady rise of blood sugar may be positively ameliorated by $1,25(OH)_2D_3$ supplementation [13].

OGTT on day 21 showed a diabetic glucose tolerance in hexavalent chromium ($K_2Cr_2O_7$) treated rats (Table 2). The results also revealed a much-improved glucose tolerance in case of $1,25(OH)_2D_3$ supplemented rats. Increased serum glucose level with a concomitant decrease of serum insulin level and resultant increased insulinogenic index in Cr (VI) treated rats indicated an impairment of glucose homeostasis (Table 3). $1,25(OH)_2D_3$ supplementation clearly showed a possible amelioratic action of $1,25(OH)_2D_3$ against hexavalent chromium induced hyperglycemia. $1,25(OH)_2D_3$ was found to be linked with increased insulin sensitivity and insulin generation, which help in better glucose homeostasis [14]. $1,25(OH)_2D_3$ deficiency-related insulin deficiencies may be due to increased levels of rise of pro-inflammatory cytokines, impairment of pancreatic beta cell functions, or even reduced glucose absorption in peripheral tissues [15].

Possible damage of pancreatic beta cells by Cr (VI) might have been partially reversed due to $1,25(OH)_2D_3$ supplementation in present study [16-17]. Study also revealed that many vitamins as antioxidants are highly protective against heavy metal induced oxidative stress in metabolically active organs [18]. These findings were further supported by our study on liver glycogen concentration in $1,25(OH)_2D_3$ supplemented Cr (VI) treated rats (group-4) against only hexavalent chromium treated rats. Depletion of liver glycogen by hexavalent chromium may be due to Cr (VI) induced increase in liver glycogenolysis and resultant increase in serum glucose levels [19]. This result is further suggestive of the breakdown of liver metabolism due to Cr (VI) induced metabolic stress [20].

Study also supported the observations on antioxidant supplementation as protective against ROS induced oxidative stress in vascular health [21]. Further, Cr (VI) may alter liver lactate dehydrogenase or pyruvate dehydrogenase activities which result in depletion of liver glycogen concentration [22].

Conclusion

$1,25(OH)_2D_3$ supplementation possibly counteracts this liver breakdown and reduces glycogen depletion in Cr (VI) treated rats. These findings clearly indicate $1,25(OH)_2D_3$ as a possible therapeutic nutrient against Cr (VI) induced alteration of glucose homeostasis.

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