1α,25 Dihydroxyvitamin D3: Therapeutic and Preventive Effects against Oxidative Stress, Hepatic, Pancreatic and Renal Injury in Alloxan-Induced Diabetes in Rats

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Summary Diabetes mellitus is a major endocrine disorder and a growing health problem in most countries which can be ameliorated by numerous bio-molecules such as 1α,25 dihydroxyvitamin D3 [1α,25(OH)2VD3]. With this in mind, the current study investigated the therapeutic and preventive effects of 1α,25(OH)2VD3 on diabetes and its side effects: toxicity in liver, pancreas and kidneys. Our results show that administration of 1α,25(OH)2VD3 in diabetic rats increased the plasmatic insulin level, favored the normal blood glucose levels and normalized the hepatic glycogen concentration. In addition, 1α,25(OH)2VD3 enhanced superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (by 207, 52 and 72%, respectively) compared to diabetic rats. It also reduced lipid peroxidation and the indices of toxicity in liver and kidneys by significantly decreasing alkaline phosphatases (PAL), aspartate and lactate transaminase (AST and ALT) activities, total and direct bilirubin, triglycerides (TG), cholesterol, creatinine, urea and iron levels in diabetic rats. Moreover, the plasmatic non-enzymatic antioxidant level of HDL-cholesterol, magnesium (Mg), calcium (Ca) and copper (Cu) increased after 1α,25(OH)2VD3 administration. The administration of 1α,25(OH)2VD3 in diabetic rats protects against alloxan-induced histological changes in pancreas. Conclusion: from these data, it is concluded that 1α,25(OH)2VD3 might be useful for the therapy and prevention of diabetes and the numerous side effects especially toxicity in liver, pancreas and kidneys and this protective effect is more obvious in our preventive experiment.

Key Words diabetes, oxidative stress, 1α,25 dihydroxyvitamin D3, pancreas, liver and kidney

Diabetes mellitus, a big public health problem, is now emerging as a deadly disease and, by the year 2030, three quarters of the world’s population (360 million persons) with diabetes will be in non-industrialized countries. But this disease is gradually becoming prevalent in developed countries too (1, 2). This disease is characterized by high levels of glucose in the blood due to the absence of insulin secretion or insulin insensitivity. An imbalance of oxidants/antioxidants in favour of oxidants contributes to the pathogenesis of diabetes. Hyperglycaemia-evoked oxidative stress plays a crucial role in the development of diabetic complications (3, 4). Current approaches to diabetes therapy involve mainly drugs enhancing insulin secretion or activity as well as inhibitors of endogenous glucose production (5).

1α,25 Dihydroxyvitamin D3 [1α,25(OH)2VD3] is an active form of vitamin D3 which exhibits various physiological actions, including the regulation of neurocognitive dysfunction and the immune system (6). It is well known that 1α,25(OH)2VD3 binds to the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily (6, 7). Clinical studies have claimed that vitamin D deficiency impairs insulin synthesis and secretion, which suggests a role in the development of diabetes (8), and it has been pointed out that the majority of patients with diabetes had vitamin D deficiency (9).

In this experimental study, we aimed to investigate the effects of 1α,25(OH)2VD3 treatments on lipid peroxidation, toxicity and the endogenous antioxidant enzyme activities in diabetic rat tissues: liver and kidneys.

MATERIALS AND METHODS

Animals and treatments. Adult male Wistar rats, weighing 179±10 g, and obtained from the Central Pharmacy, Tunisia, were used in the study. The animals were kept in an environmentally controlled breeding room (temperature: 20±2°C, humidity: 60±5%, 12-h dark/light cycle). All rats had free access to tap water and fasted overnight before blood and tissue collection. The handling of the animals was approved by the local...
Ethical Committee for the care and use of laboratory animals. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 150 mg/kg body weight (4). The feeding experiment was carried out for a period of 4 wk after the induction of diabetes (characterized by the presence of glucosuria). The rats were divided into five groups of 10 animals each.

Group 1: control animals received NaCl 0.9% (ip).

Group 2: diabetic animals received alloxan (ip).

Group 3 (therapeutic effect): animals received 5,000 IU/kg bw/d 1α,25(OH)2VD3 15 d after alloxan injection (characterized by diabetes installation characterized by the presence of glucosuria (10)).

Group 4 (preventive effect): rats received 5,000 IU/kg bw/d 1α,25(OH)2VD3 15 d before alloxan injection.

Group 5: normal rats received only 1α,25(OH)2VD3.

The vitamin D3 was administrated by the gastric gavage method. Two months after the start of the experiments (the beginning of the administration of 1α,25(OH)2VD3 in the preventive experiment), the animals were sacrificed by decapitation, and the trunk blood collected. The serum was prepared by centrifugation (1,500 × g, 15 min, 4 °C) and the kidneys and liver were removed, cleaned of fat and weighed; all these samples were stored at −80 °C until used. Pieces of pancreas were fixed in a Bouin for histological studies.

Biochemical assays. Plasma insulin was measured by RIA (Bi-insulin RIA Diagnostic, Pasteur, Paris, France). The lipid peroxidation in the liver and kidneys of control and all treated groups of animals was measured by the quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Buege and Aust (11). The activity of superoxide dismutase (SOD) in the liver and kidney of control and treated rats was assayed by the spectrophotometric method of Marklund and Marklund (12). The activities of glutathione peroxidase (GPX) and catalase (CAT) were measured by the modified method of Pagila and Valentine (13) and Aebei (14) respectively. The amount of proteins was determined by the method of Lowry et al. (15). The levels of alkaline phosphatases (PAL), aspartate and lactate transaminases (AST and ALT), total and direct bilirubin, cholesterol, triglycerides (TG), HDL-cholesterol, plasmatic and hepatic glucose, creatinine, urea, magnesium (Mg), copper (Cu), calcium (Ca)
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Statistical analyses. Data are presented as means±SD. The determinations were performed from ten animals per group and the differences were examined by the one-way analysis of variance (ANOVA) followed by the Fisher test (Stat View) and significance was accepted at $p<0.05$.

RESULTS

Body, liver and kidneys weights (Table 1)

The body, liver and kidney weights of the diabetic group were lower ($p<0.05$) than those of the controls (Table 1). After the administration of 1α,25(OH)2VD3, a significant increase of body, hepatic and renal weights was observed compared to diabetic rats and this gain was more important in preventive experiments.

Plasmatic insulin, blood glucose and hepatic glycogen Levels (Fig. 1)

This study showed a decrease in plasmatic insulin level in alloxan-treated rats by 59% compared to control rats. In addition, a significant ($p<0.001$) increase in the level of blood glucose and a decrease in hepatic glycogen rate in diabetic rats when compared to control rats were observed. However, the daily administration of the 1α,25(OH)2VD3 to diabetic rats in both preventive and therapeutic experiments significantly ($p<0.01$) increased the insulin level by 98 and 42%. This increase in insulin level in alloxan-treated rats after 1α,25(OH)2VD3 treatment caused a decreased level of blood glucose and increased hepatic glycogen level to near normal levels.

SOD, CAT and GPX in liver and kidneys (Fig. 2)

1α,25(OH)2VD3 administration to diabetic animals resulted in a marked elevation of SOD, CAT and GPX activities in hepatic and renal tissues. Additionally, in the preventive experiment, the activity of SOD, CAT and GPX was significantly higher than that in the therapeutic experiment in both tissues.

Lipid peroxidation in liver and kidneys (Fig. 3)

As shown in Fig. 3 diabetes caused progressive accumulation of lipid peroxidation in liver and kidneys after 2 mo of alloxan administration. Their content exceeded the control value by seven-fold. However, 1α,25(OH)2VD3...
treatment reduced lipid peroxidation content in hepatic and renal tissues of diabetic animals by 46 and 40% (in preventive experiments) and by 39 and 37% (in therapeutic experiments) respectively, compared to that in untreated diabetic rats.

Mg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$ and Fe$^{2+}$ levels in plasma (Table 2)

Table 2 points out that the induction of diabetes caused a significant ($p<0.001$) decrease in Mg, Ca and Cu contents and a significant increase in iron level. However, under these conditions, the administration of 1α,25(OH)2VD3 attenuated these changes and the ameliorative effect was more evident in preventive experiments.  

Hepatotoxicity indices (PAL, AST, ALT, total and direct bilirubin) (Table 3)

Table 3 shows that administration of vitamin D3 protected rats from hepatotoxicity provoked by diabetes. In fact, both in therapeutic and preventive experiments, 1α,25(OH)2VD3 significantly reduced the levels of PAL, AST, and ALT as well as total and direct bilirubin as compared with those of the unsupplemented alloxan-treated rats.

Kidney toxicity indices in plasma (creatinine, urea) (Table 4)

In diabetic rats, after 2 mo of severe diabetes induction, a state of nephropathy was observed. In fact, a significant increase in the indices of renal toxicity (increase in plasmatic creatinine and urea levels) was observed. However, 1α,25(OH)2VD3 administration to diabetic rats normalized the two indices.

Lipid assessment (Table 5)

The total cholesterol and triglyceride concentrations in the serum were significantly higher in the alloxan-induced diabetic rats than in the control rats (Table 5). The feeding with 1α,25(OH)2VD3 suppressed the increase in the total cholesterol and triglyceride levels in the serum and liver of the diabetic rats. In effect, 1α,25(OH)2VD3 supplementation in diabetic rats decreased the serum triglyceride and cholesterol levels significantly to almost the control concentration. The HDL-cholesterol concentration was also significantly lowered by the induction of diabetes; however, it was higher in the therapeutic and preventive treatments with 1α,25(OH)2VD3 compared to the other diabetic groups.

Histological findings (Fig. 4)

In pancreatic tissues, the histopathological examination reveals extensive alterations in the pancreas of alloxan-induced diabetic rats (Fig. 4A–D). The pancreas of a control rat (Fig. 4A) shows normal islets. However, the diabetic pancreas shows (Fig. 4B) an atrophy of β-cells. In preventive experiments, 1α,25(OH)2VD3 administration to diabetic rats (Fig. 4C) caused a par-
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Table 5. Total-cholesterol, triglycerides and HDL-cholesterol in the plasma of control, diabetic and 1α,25(OH)2VD3-administrated rats before and after diabetes induction.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (g/L)</th>
<th>Triglycerides (g/L)</th>
<th>HDL-cholesterol (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63±0.1</td>
<td>0.79±0.03</td>
<td>0.53±0.1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.52±0.1***</td>
<td>1.82±0.24***</td>
<td>0.34±0.05**</td>
</tr>
<tr>
<td>Diabetic + VD3_Ther</td>
<td>0.91±0.09***##</td>
<td>1.07±0.23***##</td>
<td>0.56±0.09***##</td>
</tr>
<tr>
<td>Diabetic + VD3_Prev</td>
<td>0.89±0.11***###</td>
<td>0.95±0.09***##</td>
<td>0.69±0.06***##</td>
</tr>
<tr>
<td>VD</td>
<td>0.71±0.1***###</td>
<td>0.86±0.12***###</td>
<td>0.71±0.09***###</td>
</tr>
</tbody>
</table>

Statistical analyses as in Fig. 1 legend.

Fig. 4. Effect of 1α,25(OH)2VD3 on the histological changes of rats’ pancreas by HE staining (100×). A: Normal control rats showed normal β-cells. B: Alloxan treatment elicited severe injury to pancreatic β-cells. C: Diabetic rats treated with 1α,25(OH)2VD3. 3 wk before alloxan administration (preventive experiment). A significantly reduced score of the injuries to pancreatic β-cells was observed. D: Diabetic rats treated with 1α,25(OH)2VD3. 3 wk after alloxan administration (therapeutic experiment). Low ameliorative changes compared to diabetic rats are observed.

The present work shows that the administration of alloxan leads to a high level of glucose in the blood. This high level is due to defects in insulin production shown in this study and consequently to pancreatic β-cell death and damage as shown by histological findings. The disorder of glucose metabolism in diabetes is mainly attributed to diabetic oxidative stress by several mechanisms such as increased production of free radicals especially reactive oxygen species (ROS) and proteins glycosylation (16). In fact, the increase in glucose level causes the formation of free radicals of oxygen (ROS). An oxidative atmosphere in cells is created by impairment in the functioning of endogenous antioxidant enzymes, mainly superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Hyperglycaemia also degrades non-enzymatic antioxidant defenses by allowing reactive oxygen species which damage cells and tissues (16). This study shows that hyperglycaemia is accompanied by the loss of enzyme cofactors: Cu²⁺, a metal essential for SOD activity. Besides, the administration of alloxan to rats causes a lack of magnesium and...
calcium concentration (17), trace elements which are essential for insulin secretion and activity. The decrease in both antioxidant enzymes activities and Cu²⁺ content causes an increase in lipid peroxidation in hepatic and renal tissues. These changes attack the hepatic function as shown by an increase in the level of triglycerides, LDL-cholesterol, AST, ALT, LDH and the decrease in HDL-cholesterol indices of liver damage which are also detected in human diabetic cases (18). The hyperglycemia also induces the elevation of the plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction, and all these changes decrease the body, kidney and liver weights of animals. These findings are in agreement with Igarashi et al. (19) and Duzguner and Sule Kaya (20) who reported that alloxan induced inhibition of SOD and CAT activities and increased cholesterol, triglyceride, lipid peroxidation and hepatic and renal indices of toxicity in diabetic animals.

Previous studies have shown that diabetes mellitus is associated with vitamin D deficiency (21, 22). The effect of 1α,25(OH)2D3 on normal insulin release has not yet been clarified; however, there is evidence that 1α,25(OH)2D3 directly influences insulin secretion in the β-cells through an increase in intracellular calcium (22, 23). A recent observation reveals that many of these complications are diminished upon supplementation with 1α,25(OH)2D3, and this vitamin is essential for normal insulin secretion (21). In this study, daily treatment of diabetic rats with 5,000 IU//kg bw/d 1α,25(OH)2D3 daily by gavage increased the insulin rate by 42 and 98% and this caused a decrease in blood glucose levels by 34 and 45% in therapeutic and preventive experiments respectively in comparison to the diabetic untreated group. The hypoglycemic action of 1α,25(OH)2D3 has been explained by the enhancement of insulin sensitivity and the acceleration of glucose utilization by peripheral tissues (21) and/or the increase of glycogen synthesis in liver was investigated in this study. In addition our study revealed that 1α,25(OH)2D3 administrations in diabetic rats increase the Mg²⁺ and Ca²⁺ levels in plasma which are essential metals for activation of insulin exocytosis and stimulate mitochondrial metabolism and glucose oxidation. In fact magnesium deficiency may result in disorders of tyrosine kinase activity on the insulin receptor (24), events related to the development of insulin resistance. Low serum magnesium levels are also strongly related to elevated serum concentrations of tumor necrosis factor alpha (25), suggesting that magnesium deficiency may also be involved in the development of low-grade chronic inflammation syndrome and through this pathway, with the development of glucose metabolic disorders. In this context deficiency in Mg²⁺ is associated with the development of poor metabolic control, increased free radical dependent-oxidative tissue damage, and chronic complications in patients with diabetes (24, 25). Similar to the findings with magnesium, calcium plays a pivotal role in regulating insulin secretion in pancreatic β-cells. A rise in intracellular Ca²⁺ serves as a critical trigger for insulin granule exocytosis in response to secretagogue stimulation. In fact elevation of glucose concentration in the extracellular medium of β-cells triggers the opening of voltage dependent calcium channels and the influx of Ca²⁺ into the cell and this causes an increase in insulin secretion (26, 27). Recently Wiederkehr and Wollheim (28) reported that cytosolic calcium rises are relayed into mitochondria where the ion promotes glucose oxidation and ATP production to sustain insulin secretion. The generation of other still elusive coupling factors may also depend on the mitochondrial calcium signal. Increase of mitochondrial calcium signals would be an attractive means of improving both impaired insulin secretion and defective insulin action in diabetic patients. Similarly Komoto et al. (29) and Slingerland et al. (30) reported that increase in the extracellular Mg²⁺ and Ca²⁺ concentration causes a transient increase in insulin secretion in rat and human pancreatic β-cells. The beneficial action of 1α,25(OH)2D3 in insulin secretion and activity and Cu, Mg and Ca contents in alloxan-induced diabetes in rats decrease the blood glucose level. This hypoglycemia action of vitamin D3 decreases the production of free radicals (31), the glucose autoxidation and protein glycosylation which together prevent the complication of hyperglycemia in hepatic and renal functions. Besides, the 1α,25(OH)2D3 might exert a beneficial effect as a result of their structural similarities to endogenous estrogens binding to the intranuclear estrogen receptor protein to modulate gene transcription SOD, CAT and GPX in hepatic and renal cells (4, 9). In fact, Knutu et al. (32) indicated the key role of vitamin D on estrogen biosynthesis in general and more specifically a direct regulation of the expression of the aromatase gene. Our early results (33) revealed that diabetes induced a breakdown in plasmatic estradiol level (a hormone essential in the regulation of insulin secretion and the modulation of the insulin receptors (34–36) and administration of 1α,25(OH)2D3 may prevent the decline in estrogens levels, and consequently prevent diabetes acceleration. This study also shows that 1α,25(OH)2D3 exerts hypcholesterolemic and hypolipidemic effects, best explained by increased conversion of cholesterol into bile acids like phytocolesterol (37).

Other mechanisms have been proposed to explain the antioxidant property of 1α,25(OH)2D3 as i) induction of glucose-6-phosphate dehydrogenase (G6PD) gene expression, a key antioxidant enzyme (36); ii) increase in estrogen and testosterone levels (33) which are essentials in the regulation of insulin secretion and the modulation of the insulin receptors (38, 39). All these beneficial effects of 1α,25(OH)2D3 against hepatic and renal toxicity are supported by the lower rate in lipid peroxidation and the hepatic (AST, ALT, PAL, total and direct bilirubin rate) and renal (creatinine and urea levels) levels of toxicity.

CONCLUSION

This present study clearly indicates that
1α,25(OH)2VD3 can be effective in inhibiting hyperglycemia, oxidative stress and cell damage in pancreas, liver and kidneys by enhancing insulin level and sensitivity and antioxidant capacity, and consequently the improvement of diabetes and its toxicity.

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REFERENCES


