Original Article
Effects of 1,25-dihydroxyvitamin D$_3$ on islet α cell and β cell in multiple low dose streptozotocin-induced diabetes rats

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Abstract: To study the prophylactic effects of 1,25-dihydroxyvitamin D$_3$ on α cell and β cell of pancreatic islet of diabetes rats induced by streptozotocin. Streptozotocin-induced diabetes rats were orally administrated with 1,25-dihydroxyvitamin D$_3$ every day for 4 weeks. The incidence and Glucose AUC of diabetes in the 1,25-dihydroxyvitamin D$_3$ group was much less than in STZ group, while insulin AUC in the 1,25-dihydroxyvitamin D$_3$ group was larger than that in the STZ group. TNFα and BAX expression in 1,25-dihydroxyvitamin D$_3$ group were declined in comparison with those in STZ group. Therefore, 1,25-dihydroxyvitamin D$_3$ may prevent Sprague-Dawley rat from diabetes induced by streptozotocin probably through downregulating the expression TNFα and BAX, which may lead to enhance the islet apoptosis and hyperglycemia.

Keywords: 1,25-dihydroxyvitamin D$_3$, diabetes, islet cell

Introduction

Epidemiological studies show that the incidence of Nordic type 1 diabetes mellitus (T1DM) is the highest in the world, which is presumably related to less sunshine in Northern Europe, whereas human plasma vitamin D (VD) level is directly related to the amount of sunshine [1]. Other studies show that VD deficiency significantly increases the incidence of T1DM while supplementation of VD during infancy reduces the incidence of T1DM. Both in vitro and in vivo studies have confirmed that 1,25(OH)$_2$D$_3$ has immunomodulatory effect and can prevent T1DM [2].

The pathogenesis of T1DM is positively related to autoimmunity. The main form of β-cell destruction in DM is β-cell apoptosis in pancreatic islets. In rats with STZ-induced DM, pancreatic β-cell apoptosis is related to STZ toxicity and may also be related to STZ-induced immune injury [3]. Among which, T-cells and the produced cytokines play important roles in the pathogenesis of T1DM. Tumor necrosis factor α (TNFα), a cytokine produced by T-cells, can induce pancreatic β-cell apoptosis through mu-ltiple pathways and is regulated by a variety of related genes [4]. Currently, there are limited studies on the relationship between bax/bcl-2 and pancreatic β-cell apoptosis. The bax (apoptosis-promoting gene) exists in homodimer (bax/bax) or forms heterodimer (bax/bcl-2) with bcl-2 (cell longevity gene), its ratio determines apoptosis rate. Although the immunomodulation of 1,25(OH)$_2$D$_3$ and its prevention of T1DM have been confirmed, the mechanism is not fully understood and lacks cytokine and apoptosis data for confirmation [5]. This experimental study intended to examine the roles of 1,25(OH)$_2$D$_3$ in the prevention of STZ-induced DM in rats, to investigate the roles of TNFα, bax and bcl-2 mRNA and determine their relationship, so as to provide experimental evidence for the prevention of human T1DM.

Materials and methods

Experimental animals

Chinese PLA in Beijing. The animals had free access to water and food (standard pellet feeds), 12 hr lighting daily.

Table 1. RT-PCR primer sequence and product size

Target gene	Sense primer	Anti-sense primer	Length (bp)
Bcl-2	5'AGCGTCAACAGG GAGATGTGA3'	5'TGAGCAGCGTCTTCA GAGACA3'	184 bp
Bax	5'GGTTTCATCCAGGAT CGAGCA3'	5'TCAGCAAACATGTCA GCTGCC3'	221 bp
GAPDH	5'TGAAGGTCGGTGTCAAC GGATTTGGC3'	5'CATGTAGGCCATGAG GTCCACCAC3'	983 bp
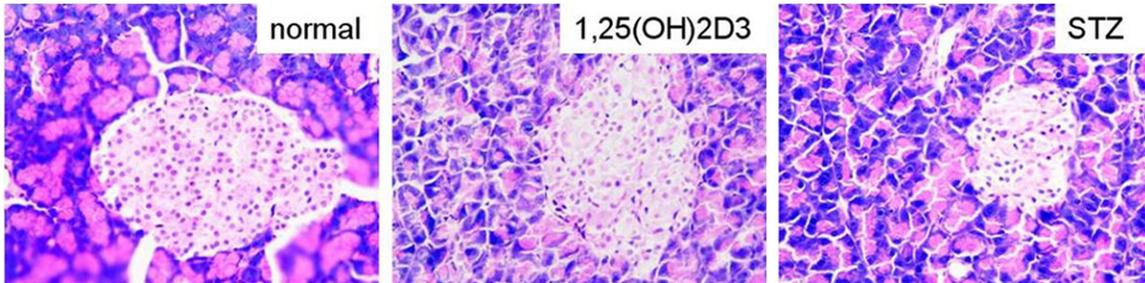
Figure 1. Pathological examination of pancreatic islets in each group.

Table 2. α cells/mm^2 and β cells/mm^2

	α cells/mm^2	β cells/mm^2
Normal	3.7045±0.536	13.390±3.362
STZ	5.503±0.567*,#	4.032±0.762**,#
1,25(OH)$_2$D$_3$	4.072±0.699	6.104±1.356**

*compared with normal group, P<0.05, **compared with normalgroup, P<0.01, #compared with 1,25(OH)$_2$D$_3$ group, P<0.05.

Grouping and treatment

The animals were randomized into 3 groups by body weight.

Normal control group (group A, n=8): 0.05 ml/day of peanut oil was administered through intragastric gavage for 5 weeks, then citrate buffer was injected for 2 weeks, then the animals were sacrificed.

STZ control group (group B, n=10): 0.05 ml/day of peanut oil was administered through intragastric gavage for 5 weeks, 0.5 mL complete Freund adjuvant (CFA) was injected in rats, then STZ was injected in rats with 30 mg/kg on next day and continuous 6 weeks. Blood glucose was randomly measured in 72 hr after modeling; the modeling was considered to be successful if blood glucose was ≥14.0 mmol/L.

1,25(OH)$_2$D$_3$ group (n=10): 1,25(OH)$_2$D$_3$ (1 μg/kg/day)was dissolved in 0.05 ml peanut oil for intragastric gavage for 4 weeks. 0.5 mL complete Freund adjuvant (CFA) was injected in rats, then STZ was injected in rats with 30 mg/kg on next day and continuous 6 weeks.

For 1 and 2: In 1 week after modeling, glucose tolerance test and insulin release test were conducted to calculate and compare blood glucose and insulin AUC between all groups. (Rat insulin ELISA kit was purchased from Mercodia, USA. For 3: Blood concentrations of 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ were determined with radioimmunoassay (Kit was purchased from DiaSorin, USA).

Immunohistochemistry assay

HE staining method was used to determine the pathological changes of pancreatic islet cells, the area of occupied pancreatic islets and TNFα expression in each group.

Azocarmine Azon-Gomori simplified Bloom's pancreatic islet cell staining technique was used to compare the differences in pancreatic α-cell and β-cell counts between these groups.

Transmission electron microscopy and scanning electron microscopy were used to observe the ultrastructural changes in pancreatic islet cells.

RT-PCR assay

RT-PCR method was used to detect pancreatic BAX and BCL-2 gene expression. Trizol (Invetrogen) was used to extract total RNA; the OD260/OD280 ratio determined with UV spectrophotometry was 1.9, indicating high RNA purity. In the subsequent reverse transcription reaction, 10 μg of total RNA was used; 1 μL Olig(dT)$_{18}$ (Invetrogen) and 6 μL of 1‰ DEPC water were added and mixed evenly; immedi-
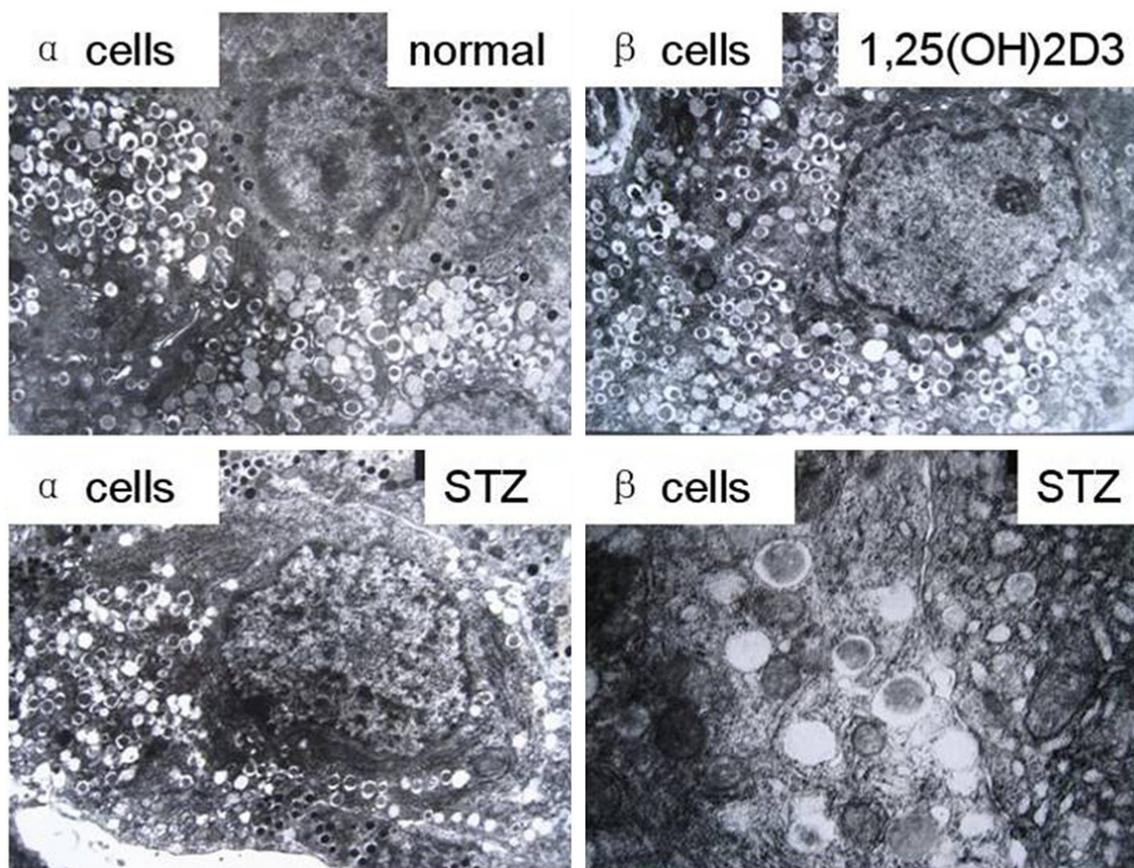
Figure 2. Electron microscopic results of pancreatic islets in each group.

ately after incubation in a 70°C water bath for 5 min, the samples were transferred into an ice bath; then, 5 μL of 5× buffer solution, 2.5 μL of 10 m MdNTP (Takara Biotechnology (Dalian) Co. Ltd.), 1.0 μL RNase inhibitor (Takara Biotechnology (Dalian) Co. Ltd.), 1.0 μL reverse transcriptase (Promega) and 6.5 μL of 1‰ DEPC water were added and mixed evenly before incubation in a 37°C water bath for 1hr, followed by incubation in a 70°C water bath for 5 min. Finally, target gene amplification (PCR) was carried out; see **Table 1** for primer sequence and product size. PCR reaction conditions: pre-denaturation at 94°C for 5 min, followed by 94°C 40 s, 72°C 50 s, 59°C 40 s and 72°C 50 s, totally 35 cycles; then 72°C for 5 min to end the extension.

Results

Pathological examination of pancreatic islets in each group

Light microscopy: Normal control group: under low-power field, there were varying sizes of scattered pancreatic islet tissues with clear edge and regular shape. Under high-power field, large cell body with abundant cytoplasm was observed; the cell boundaries were clear with visible intercellular sinusoidal capillaries. 1,25(OH)$_2$D$_3$ group: under low-power field, pancreatic islet counts slightly decreased with normal morphology. Under high-power field, the cells were partially similar to that in normal control group, but the cytoplasm showed slight swelling and degeneration. STZ group: under low-power field, pancreatic islet counts decreased with irregular morphology. Under high-power field, pancreatic islet cell counts significantly decreased; the cell body shrank with blurred boundary and some fibrosis; some cytoplasm showed eosinophilic dark staining; some nuclei were basophilic with karyopyknosis and karyorrhexis (**Figure 1**).

Changes of α-cell counts in each group (represented as α-cell counts/pancreatic islet area). The α-cell counts/mm^2 was higher in STZ group than in control group and 1,25(OH)$_2$D$_3$ group,

Table 3. Effect of blood glucose AUC and insulin AUC

	Blood glucose AUC(mmol.h/L)	Insulin AUC (μg.h/L)
Normal	8.445±1.236	1.8558±0.362
STZ	31.183±4.567[**,#]	0.4717±0.162[**,#]
1,25(OH)$_2$D$_3$	21.301±4.699[**]	1.1046±0.356[**]

[**]compared with normal group, P<0.01, [#]compared with 1,25(OH)$_2$D$_3$ group, P<0.05.

Table 4. Level of 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ with treatment

	1,25(OH)$_2$D$_3$ (pg/mL)	25(OH)D$_3$ (ng/mL)
Normal	199.67±38.33[**]	120.36±23.56
STZ	181.39±20.82[**]	116.32±12.36
1,25(OH)$_2$D$_3$	341.82±43.11	111.18±19.86

[**]compared with 1,25(OH)$_2$D$_3$ group, P<0.01.

Table 5. Correlation on 1,25(OH)$_2$D$_3$, blood glucose AUC and insulin AUC

	Correlation factor (r)	P value
Blood glucose AUC	-0.562	0.024
Insulin	0.517	0.04

P<0.05. Changes in β-cell counts in each group (represented as β-cell counts/pancreatic islet area). β-cell counts/mm^2 were significantly lower in each STZ treatment group than in normal control group (F=31.010, P=0.000), among which, β-cell counts were significantly lower in STZ group than in 1,25(OH)$_2$D$_3$ group (P=0.046), suggesting that 1,25(OH)$_2$D$_3$ was able to protect pancreatic β-cells in DM rats from STZ-induced injuries. Therefore, 1,25(OH)$_2$D$_3$ had certain protective effect on pancreatic β-cells (**Table 2**).

Electron microscopic results

Pancreatic β-cells: majority of cells in normal rat pancreatic islets are β-cells. The round or oval nuclei contained visible medium-sized nucleoli and had small projections and invaginations on the surface. Heterochromatins were mostly distributed by the nuclear envelope; only a few heterochromatins scattered among the significant cytoplasmic characteristics was that the cytoplasm was full of insulin secretory granules. These round or oval encapsulated granules had the following morphologies: 1.

There was transparent halo (gap) within the membrane and the core contained high electron-dense substance; 2. They were similar to the above granules, but the core contained medium electron-dense substance; 3. The core contained encapsulated low electron-dense substance without halo structure between the membrane and the core. These 3 types of granules were all insulin granules in different states with a mean diameter of 202-333 nm.

The cytoplasm contained a few smaller darker mitochondria with fuzzy cristae. Fewer rough endoplasmic reticulum was in fine tubular shape or small vesicular shape due to slight dilation. The Golgi complex was also small and the saccules were slightly stretched. These organelles were distributed among the secretory granules and occupied less cytoplasmic space.

The cytoplasm contained small heaps of or scattering glucagon secretory granules that were much lower in number than insulin granules in β-cells. In the encapsulated granules, halo between the membrane and the core was visible in some granules but not in others. All cores contained high electron-dense substances. The diameter of these granules was approximately 148-253 nm. The cytoplasm contained abundant free ribosomes and polyribosomes; despite the smaller mitochondria and rough endoplasmic reticulum, their number was slightly higher than in β-cells.

Significant changes in the cytoplasm involved the secretory granules, including significantly reduced number of insulin secretory granules; the cytoplasm full of insulin secretory granules in normal rat pancreatic β cells was not observed. In some cells, the insulin secretory granules piled or scattered in the cytoplasm; these granules have largely lost the morphological characteristics of normal granules, specifically, most granule membrane and cores were completely dissolved to form electron-transparent capsule-free vesicles; very few secretory granules with normal structure were observed, but the volume was small. Meanwhile, in some other cells, the cytoplasm contained sparse secretory granules or no secretory granules at all, which was completely replaced with a large number of dilated rough endoplasmic reticulum storing more medium electron-dense substance. In most cells containing secretory granules, the organelles degenerated significantly.

Table 6. mRNA level of TNFα, Bax-2 and Bcl-2 in rat pancreas

	N	TNFα	Bax	Bcl-2	Bax/Bcl-2
Normal	8	1.19±0.23	0.35±0.09	1.00±0.34	0.39±0.18
STZ	10	4.16±1.20[**,ΔΔ]	0.90±0.32[**,Δ]	0.59±0.18[**]	1.32±0.31[**,Δ]
1,25(OH)$_2$D$_3$	10	2.50±0.63[*,Δ]	0.61±0.21[*]	0.72±0.25[*]	0.87±0.29[**]

[*]compared with normal group, P<0.05; [**]compared with. normal group, P<0.01; [Δ]compared with 1,25(OH)$_2$D$_3$ group, P<0.05; [ΔΔ]compared with 1,25(OH)$_2$D$_3$ group, P<0.01.

There were fewer mitochondria; most mitochondrial cristae and matrix were dissolved, even severe enough to form mitochondrial vacuoles (inner cristae completely disappeared). The rough endoplasmic reticulum was transparent vesicle in shape. Golgi complex saccules and vesicles also dilated with transparent cisterna. A few small lysosomes were visible in the cytoplasm.

α-cell hyperplasia was significant; 1 β-cell was surrounded by several α-cells, but these α-cells had normal morphology without significant abnormality. (The structure was basically similar to the normal control). Cytoplasmic secretory granules significantly increased when compared with normal control group; densely distributed secretory granules were readily visible. Mild degeneration of the organelles such as mildly dilated rough endoplasmic reticulum and membrane dissolution were observed; mitochondrial cristae and matrix mildly dissolved.

Pancreatic β-cells: the ultrastructure of β-cells was similarly between this group and normal control group. The cytoplasm was full of abundant secretory granules and the structure of most granules was similar to normal control group. There were 3 different states of granules, but dissolved small secretory granules were rare. The mitochondria in the cytoplasm were small and dark without clear cristae structure. The rough endoplasmic reticulum slightly dilated to form vesicular shape. Golgi complex also slightly dilated (**Figure 2**).

Changes in blood glucose AUC and insulin AUC in each group of rats

Blood glucose AUC was higher while insulin AUC was lower in STZ treatment group than in normal control group (P<0.01). Blood glucose AUC was lower while insulin AUC was higher in 1,25(OH)$_2$D$_3$ group than in STZ group (P<0.05), suggesting that 1,25(OH)$_2$D$_3$ played certain roles in protecting pancreatic β-cells in rats with STZ-induced DM. Effects on DM rat model-

ing: 10 animals in STZ group were successfully modeled; only 6 of the 10 animals in 1,25(OH)$_2$D$_3$ group were modeled. When exact probability was calculated with the 2×2 table, the difference between STZ group and 1,25(OH)$_2$D$_3$ group had statistical significance (P=0.046). Blood glucose AUC was significantly higher (P=0.00) while insulin AUC was lower (P<0.05) in STZ treatment group B and C than in normal control group; blood glucose AUC was significantly lower (P=0.01) while insulin AUC was higher (P=0.027) in group C than in group B, suggesting that 1,25(OH)$_2$D$_3$ played certain roles in protecting pancreatic β-cells in rats with STZ-induced DM, alleviated STZ-induced pancreatic β-cell injuries, and tapered the amplitude of declining insulin secretion (**Table 3**).

Blood 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ level: in 4 weeks after 1,25(OH)$_2$D$_3$ was administered, serum 1,25(OH)$_2$D$_3$ level was significantly higher in group C than in other 2 groups (P<0.01), but there was no difference in 25(OH)D$_3$ level between these groups.

Correlation analysis between blood 1,25(OH)$_2$D$_3$ level and blood glucose AUC and insulin AUC: 1,25(OH)$_2$D$_3$ was positively correlated with insulin AUC and negatively correlated with blood glucose AUC (**Tables 4** and **5**).

Expression of pancreatic BAX-mRNA, BCL-2-mRNA and TNFα

When compared with normal control group, pancreatic Bax-mRNA and TNFα expression increased in all STZ treatment groups, P<0.05; when compared with STZ group, Bax-mRNA and TNFα expression was down-regulated in 1,25(OH)$_2$D$_3$ group, the difference had statistical significance. Bcl-2mRNA level was lower in all STZ treatment groups than in normal control group, the difference had statistical significance, but there were no differences in Bcl-2mRNA level between STZ treatment groups. Bax/Bcl-2 level was significantly lower in 1,25(OH)$_2$D$_3$ group than in STZ group, the differences had statistical significance (P=0.004), suggesting that Bax and Bcl-2 gene played important roles in apoptosis in rats with STZ-induced DM; while 1,25(OH)$_2$D$_3$ tapered the rising amplitude of Bax-mRNA, and TNFα expres-

Table 7. mRNA level of Bax-2 and Bcl-2 in rat islets of langerhans

	R	T	P	Regression equation
Bax	0.501	2.776	0.011	Bax =0.37+0.10TNFα (F=7.718, P=0.011)
Bcl-2	-0.306	-0.078	0.137	
Bax/Bcl-2	0.586	3.340	0.002	Bax/Bcl-2=0.43+0.17TNFα (F=12.006, P=0.002)

sion level and Bax/Bcl-2 ratio in rats with STZ-induced DM, providing protection for rats with DM accordingly (**Table 6**). Correlation analysis of islet TNFα, pancreatic Bax, Bcl-2mRNA expression and their ratio (**Table 7**): islet TNFα level was positively correlated with pancreatic Bax mRNA expression and Bax/Bcl-2, but unrelated to Bcl-2 mRNA expression.

Discussion

It is well known that the patients with T1DM had hyperglycemia accompanied by hypoinsulinemia while glucagon secretion significantly increased. In patients with T1DM, plasma glucagon level does neither decrease with elevated blood glucose and nor increase with decreased blood glucose, but rather maintaining at a certain level. However, plasma glucagon significantly increases after intravenous infusion of arginine or consumption of protein meals. Scholars have believed for many years that significant increase in plasma glucagon is the body's response to reduced insulin secretion in pancreatic islet so that the pancreatic α-cell counts will presumably increase, but so far, the basic studies in this aspect is very limited [6-8].

Many studies showed that 1,25(OH)$_2$D$_3$ could prevent the occurrence of T1DM through immunomodulation, but the exact mechanism is still unknown. The SD rat DM model induced by repeated intraperitoneal injections of low-dose STZ is similar to the pathogenesis of human T1DM [9]. This study showed: (1) When compared with normal control group and 1,25-(OH)$_2$D$_3$ group, pancreatic β-cell counts in STZ group significantly decreased, but pancreatic α-cell counts/mm^2 in STZ group significantly increased (P<0.05); electron microscopic results further confirmed that not only β-cell counts were lower in STZ group than in normal control group and 1,25(OH)$_2$D$_3$ group but also the secretory granules in β-cells significantly decreased; when compared with normal control group and 1,25(OH)$_2$D$_3$ group, both α-cell counts and secretory granules significantly increased in STZ groups, suggesting that

1,25(OH)$_2$D$_3$ played significant roles in protecting pancreatic β-cells to attenuate the reduction of their counts [10]. Immunohistochemical assay results confirmed the deposition of IgA, IgG and IgM in pancreatic islets of rats with STZ-induced SD, suggesting the presence of immune injury of pancreatic islets (unpublished data). As an immunomodulatory hormone, 1,25(OH)$_2$D$_3$ can prevent STZ-induced immune response to a certain extent so that the pancreatic β-cells can obtain certain degree of protection [11].

As endocrine cells, pancreatic β-cells secrete insulin to exert actions at least in following 3 aspects: 1. Endocrine: once secreted, insulin is delivered to the whole body with blood circulation to regulate sugar, fat and protein metabolism in the body and meet the basic physiological needs of the body; 2. Autocrine: once secreted, insulin protects and regulates the secretory β-cells [12]. This role is significant is lower animals. Insulin's protective effect on β-cells has undoubtedly provided a strong theoretical basis for our clinical use of insulin intensive therapy. 3. Paracrine: insulin exerts certain inhibitory effect on the growth and differentiation of adjacent pancreatic α-cells. When insulin concentration around pancreatic β-cells increases, pancreatic α-cell growth and differentiation are effectively inhibited; when insulin level sharply declines, pancreatic α-cell growth and differentiation are out of control, resulting in significant elevation of blood glucagon and inevitable hyperglycemia in patients [13].

Another possibility is that pancreatic α-cells and β-cells are derived from common stem cells. Under the action of STZ, the transformation of their common stem cells is in favor of α-cells, leading to reduced generation of pancreatic β-cells. The protective effect of 1,25(OH)$_2$D$_3$ on T1DM may be mediated by its immunomodulation so that more stem cells transform into pancreatic β-cells to protect pancreatic β-cells and prevent the occurrence of DM [14].

Since little is known about the roles of 1,25-(OH)$_2$D$_3$ besides the bone, there should be plenty room and ideas for further studies. Many aspects of its roles in upper respiratory tract infection are yet to be elucidated. We had ever predicted the presence of hypocalcemia in patients with SARS, which was confirmed during the rescue process of these patients. In fact, decreased 1,25(OH)$_2$D$_3$ level was the cause of hypocalcemia. The reasons may be VD deficiency due to inadequate exposure to sunshine or inadequate 1,25(OH)$_2$D$_3$ synthesis due to renal tubular damage prior to infection, resulting in insufficient intestinal absorption of calcium and phosphorus, and decreased blood calcium level; consequently, the patients become susceptible to respiratory infection, while infection is the source of a variety of autoimmune diseases. The author opened up a new way of thinking for the treatment and prevention of DM in order to prevent pancreatic β-cells from injuries, maintain their normal secretory function, and avoid the occurrence of hyperglycemia [15].

Once T1DM occurs, timely insulin therapy and even intensive insulin therapy are unable to reverse the subsequently emerged chronic complications and the resulted high mortality. Therefore, the prevention of T1DM is particularly important.

Despite the significant species difference between animal disease model and human disease, animal studies can avoid many uncontrollable factors and directly reveal pathological basis of disease. The commonly used methods to replicate T1DM animal models mainly include induced model and spontaneous model. DM model induced by multiple intraperitoneal injection of low-dose STZ not only resembles the etiology and pathogenesis of T1DM but also is economic and feasible [16].

Conventional immunosuppressants such as cyclosporin A, azathioprine and other were once used for the treatment of T1DM, but no satisfactory results are obtained yet. In addition, the above drugs also had some side effects. Therefore, we try to find such a drug that can induce immune tolerance of foreign antigen for pancreatic β-cells and protect pancreatic β-cells from autoimmune injury at the same time; ideally, the drug can also enhance insulin secretion, but the most important characteristic is the lack of serious toxic and side effects.

Epidemiological studies showed that VD was related to the incidence of T1DM; other studies also confirmed that 1,25(OH)$_2$D$_3$ had some potential actions yet to be elucidated besides the known effects on bone metabolism. The results of this study confirmed that 1,25(OH)$_2$D$_3$ played certain protective roles in STZ-induced DM. Past studies showed that 1,25(OH)$_2$D$_3$ exerted its effects by restoring imbalanced Th1/Th2 and inducing immune tolerance. This study confirmed that ① 1,25(OH)$_2$D$_3$ down-regulated pancreatic TNF-α expression, BAX-mRNA expression and BAX/BCL-2 ratio in STZ-induced DM; ② In 1,25(OH)$_2$D$_3$ group, BAX-mRNA expression and BAX/BCL-2 ratio were related to TNF-α expression; 1,25(OH)$_2$D$_3$ was able to inhibit pancreatic TNF-α expression; therefore, 1,25(OH)$_2$D$_3$ possibly inhibits TNF-α expression to down-regulate BAX-mRNA expression and decrease BAX/BCL-2 ratio in order to resist pancreatic β-cell apoptosis and protect pancreatic β-cells in rats with DM induced by repeated intraperitoneal injection of small-dose STZ.

Disclosure of conflict of interest

None.

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References

[1] Esquiaveto-Aun AM, De Mello MP, Paulino MF, Minicucci WJ, Guerra-Júnior G, De Lemos-Marini SH. A new compound heterozygosis for inactivating mutations in the glucokinase gene as cause of permanent neonatal diabetes mellitus (PNDM) in double-first cousins. Diabetol Metab Syndr 2015; 7: 101.

[2] Thijs W, Janssen K, van Schadewijk AM, Papapoulos SE, le Cessie S, Middeldorp S, Melissant CF, Rabe KF, Hiemstra PS. Nasal levels of antimicrobial peptides in allergic asthma patients and healthy controls: differences and effect of a short 1,25(OH)2 Vitamin D3 treatment. PLoS One 2015; 11: e0140986.

[3] Chen X, Rao SQ, Gao BH, Jiang ZQ. Effect of early vitamin D supplementation on asthma and the possible mechanisms. Genet Mol Res 2015; 4: 14136-14143.

[4] Cao KG, Fu CH, Li HQ, Xin XY, Gao Y. A new prognostic scale for the early prediction of ischemic stroke recovery mainly based on traditional Chinese medicine symptoms and NIHSS score: a retrospective cohort study. BMC Complement Altern Med 2015; 1: 407.

[5] Kerr HR, Hatipoglu B, Krishnamurthi V. Pancreas transplant for diabetes mellitus. Cleve Clin J Med 2015; 11: 738-744.

[6] Weber-Wulff D. In-dental office screening for diabetes mellitus using gingival crevicular blood: Retraction. J Int Soc Prev Community Dent 2015; 5: 432.

[7] Wafa S, Nakhla M. Improving the transition from pediatric to adult diabetes healthcare: a literature review. Can J Diabetes 2015; 6: 520-528.

[8] Yang M, Xu J, Yu J, Yang B, Gan H, Li S, Li X. Anti-inflammatory effects of 1,25-dihydroxyvitamin D3 in monocytes cultured in serum from patients with type 2 diabetes mellitus and diabetic nephropathy with uremia via Toll-like receptor 4 and nuclear factor-κB p65. Mol Med Rep 2015; 6: 8215-8222.

[9] Suwita BM, Laksmi PW, Wijaya IP. Extended dual antiplatelet for diabetic elderly patients after drug-eluting stent implantation: an evidence-based clinical review. Acta Med Indones 2015; 3: 253-264.

[10] Rahmanian K, Shojaei M, Sotoodeh Jahromi A, Madani A. The association between pre-diabetes with body mass index and marital status in an iranian urban population. Glob J Health Sci 2015; 4: 48954.

[11] Chen S, Zhu J, Zuo S, Ma J, Zhang J, Chen G, Wang X, Pan Y, Liu Y, Wang P. 1,25(OH)$_2$D$_3$ attenuates TGF-β1/β2-induced increased migration and invasion via inhibiting epithelial-mesenchymal transition in colon cancer cells. Biochem Biophys Res Commun 2015; 130-135.

[12] Pande VV, Chousalkar KC, Bhanugopan MS, Quinn JC. Super pharmacological levels of calcitriol (1,25-(OH)$_2$D$_3$) inhibits mineral deposition and decreases cell proliferation in a strain dependent manner in chicken mesenchymal stem cells undergoing osteogenic differentiation in vitro. Poult Sci 2015; 11: 2784-2796.

[13] Jain D, Weber G, Eberhard D, Mehana AE, Eglinger J, Welters A, Bartosinska B, Jeruschke K, Weiss J, Päth G, Ariga H, Seufert J, Lammert E. DJ-1 protects pancreatic beta cells from cytokine- and streptozotocin-mediated cell death. PLoS One 2015; 9: e0138535.

[14] Pillai R, Paglialunga S, Hoang M, Cousteils K, Prentice KJ, Bombardier E, Huang M, Gonzalez FJ, Tupling AR, Wheeler MB, Joseph JW. Deletion of ARNT/HIF1β in pancreatic beta cells does not impair glucose homeostasis in mice, but is associated with defective glucose sensing ex vivo. Diabetologia 2015; 12: 2832-2842.

[15] López-Acosta JF, Villa-Pérez P, Fernández-Díaz CM, Román Dde L, Díaz-Marrero AR, Cueto M, Perdomo G, Cózar-Castellano I. Protective effects of epoxypukalide on pancreatic β-cells and glucose metabolism in STZ-induced diabetic mice. Islets 2015; 2: e1078053.

[16] Chen KC, Sung SY, Lin YT, Hsieh CL, Shen KH, Peng CC, Peng RY. Benign prostatic hyperplasia complicated with T1DM can be alleviated by treadmill exercise-evidences revealed by the rat model. BMC Urol 2015; 1: 113.