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Original Article

Effects of 25-hydroxyvitamin D on bone microstructure in T2MD rats

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Abstract

Objective: This study aims to explore the effects of 25-hydroxyvitamin D on the bone microstructure of rats with type 2 diabetes mellitus (T2MD). **Methods:** 40 male Wistar rats were randomly selected for T2MD modeling and injected with streptozotocin solution. The rats in the control group (n=19) were fed with common feed. 25-hydroxyvitamin D was injected into rats with successful modeling results (Treatment group, n=15). The remaining rats were considered as the model group (n=16). The enzyme-linked immunosorbent assay was adopted to determine bone gla protein (BGP) and tartrate-resistant acid phosphatase (TRACP), and an X-ray bone densitometer were applied to observe the vertebral sections. **Results:** The activity levels of blood glucose, triglyceride, total cholesterol and TRACP in the model group were higher than those in the treatment group and the control group ($p<0.01$), while serum calcium, phosphorus, BGP, ALP, and glycosylated hemoglobin, various indicators of rats in the model group were lower than those in the treatment group and the control group ($p<0.05$). **Conclusions:** It is feasible to treat rats with T2MD with 25-hydroxyvitamin D, which can maintain the integrity of bone microstructure and increase the bone health.

Keywords: 25-hydroxyvitamin D, T2MD, Rat Model, Bone Microstructure, Effect Analysis

Introduction

Diabetes mellitus (DM), primarily triggered by hyperglycemia, is a metabolic disease whose incidence rate is extremely high in the aged population. The deteriorated DM easily gives rise to such malignant diseases as cardiovascular complications, nephrosis, and neurasthenia[1,2]. Osteoporosis is also one of the most common diseases in the aged population, which is often accompanied by diseases such as DM and hypertension[3]. DM is an intractable disease that is easily diagnosed but difficultly cured, so drugs often need to be applied for a long-term, and the intake of carbohydrates of patients need to be controlled for clinical treatment[4]. 25-hydroxyvitamin D can treat DM and supplement vitamin D required by the patient's body, which has been put into practical use in clinical practice[5]. Nevertheless, there is no study identifying whether 25-hydroxyvitamin D is affecting the bone microstructure in the treatment of DM. Hence, this study analyzed the effects of 25-hydroxyvitamin D on the bone microstructure of rats by the establishment of the rat model of type 2 DM (T2MD). At the same time, it aims to study whether 25-hydroxyvitamin will result in osteoporosis in DM patients and further identify the correlation between DM and the bone microstructure, thus providing reference and guidance for clinical practice in the future.

Materials and methods

Laboratory animals and main reagents

A total of 60 male Wistar rats with the initial body weight of 160-220 g were provided by the Laboratory Animal Center of Central South University. Vitamin D drops were

The authors have no conflict of interest.

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Table I. Comparisons of the detection results of blood glucose, insulin, glycosylated hemoglobin, triglyceride and total cholesterol.

	n	Blood glucose (mol/L)	Insulin (mmol/L)	Glycosylated hemoglobin (%)	Triglyceride (mol/L)	Total cholesterol (mol/L)
Model group	16	18.64±2.34	25.15±6.59	4.12±0.15	2.04±0.16	6.85±0.64
Treatment group	15	6.32±1.71*	39.55±6.91*	7.44±0.76*	1.24±0.26*	5.01±0.47*
Control group	19	5.21±0.54*	45.62±8.34*	8.64±1.28*	0.92±0.11*	4.57±0.81*
F		335.51	34.29	114.92	8.60	54.67
p		<0.01	<0.01	<0.01	<0.01	<0.01
*Note: * p<0.05 versus model group.*						

Table II. Comparisons of the detection results of serum calcium, phosphorus, ALP, BGP and TRACP.

	n	Serum calcium (mol/L)	Serum phosphorus (mol/L)	ALP (mol/L)	BGP (mg/L)	TRACP (U/L)
Model group	16	1.04±0.09	1.27±0.16	118.24±29.63	1.01±0.21	22.51±5.66
Treatment group	15	2.01±0.12*	2.19±0.25*	487.52±41.31*	1.94±0.39*	12.42±3.11*
Control group	19	2.84±0.15*	2.65±0.38*	564.17±51.36*	2.32±0.41*	10.92±2.72*
F		908.31	102.63	527.34	62.25	41.35
p		<0.01	<0.01	<0.01	<0.01	<0.01
*Note: *p<0.05 versus model group.*						

produced by Sinopharm Group (Xiamen) Co., Ltd., citrate buffer by Chongqing Chuandong Chemical (Group) Co., Ltd., and streptozotocin solution by Sigma, USA.

Modeling methods and grouping

A total of 60 rats were raised in an environment with humidity of 35-55% at 26°C±0.5°C and were randomly divided into the model group (n=40) underwent T2MD modeling and the control group (n=20) fed with normal feed. Rats in the model group were fed with high-sugar feed (20% sucrose, 20% lard, 2.0% cholesterol, 1.0% cholate and 57% normal diet) for 5 consecutive weeks, followed by solid instead of liquid fasting. During the solid fasting, 10 mg/mL streptozotocin solution prepared by 0.1 mol/L citrate buffer through ice bath was injected every other 24 h. After the injection twice, the rats were continued to be fed with high-fat feed for 4 weeks. Then the fasting blood glucose of the rats was measured, and the fasting blood glucose >12.2 mol/L represented for successful modeling. A half (n=20) of randomly selected rats with successful modeling were injected with 25-hydroxyvitamin D (15 mg/kg/d) every day for 7 consecutive days, which were taken as the treatment group, and the remaining rats (n=20) were considered as the model group.

Experimental methods

On the 12th week, all rats were sacrificed via cervical dislocation, and 4 mL of heart blood was extracted from each group of rats using anticoagulant tubes. The blood was divided into two parts using the anticoagulant tubes. One part was placed at room temperature for 10-20 min, and serum was removed through centrifugation for 5 min using a centrifuge (3000 rp/m). After that, a GF-2 automatic biochemical analyzer was applied to detect serum glucose, insulin, glycosylated hemoglobin, triglyceride, total cholesterol, calcium, phosphorus and alkaline phosphatase (ALP). The other part was subjected to detection of bone gla protein (BGP) and tartrate-resistant acid phosphatase (TRACP) in the serum via the enzyme-linked immunosorbent assay (ELISA). After the rats were anesthetized, they received the whole body scan using an X-ray bone densitometer, to conduct a comparative analysis of the major backbone parts such as the lumbar vertebra, femur, and tibia. Lumbar vertebrae of the rats were peeled off, repeatedly washed with normal saline and stained, and the difference in the microstructure of the lumbar vertebra sections among each group of rats was observed under a microscope.

Statistical methods

Statistical Product and Service Solutions (SPSS) 23.0 software was used for data analysis. Measurement results were all expressed as ($\bar{X}$ ±s). The analysis of variance was used for intergroup comparisons and statistical significance was set at p<0.05.

Table III. Comparison of the density of each part detected via X-ray (g/cm^2).

	n	Lumbar vertebra	Femur	Tibia	Triglyceride (mol/L)	Total cholesterol (mol/L)
Model group	16	0.128±0.023	0.091±0.020	0.087±0.013	2.04±0.16	6.85±0.64
Treatment group	15	0.177±0.015*	0.150±0.012*	0.116±0.009*	1.24±0.26*	5.01±0.47*
Control group	19	0.183±0.011*	0.159±0.009*	0.124±0.004*	0.92±0.11*	4.57±0.81*
F		53.37	112.32	75.57	8.60	54.67
p		<0.01	<0.01	<0.01	<0.01	<0.01
*Note: *p<0.05 versus model group.*						

Table IV. Observation of bone microstructure ($\bar{X}$±s).

	n	Bone mineral density (%)	Trabecular thickness (μm)	Trabecular number	Trabecular separation degree (μm)
Model group	16	3.51±2.01	50.77±15.43	0.47±0.27	3248.66±312.71
Treatment group	15	5.26±1.57*	61.16±12.64*	1.15±0.33*	1099.94±306.58*
Control group	19	6.52±2.54*	68.43±18.24*	1.24±0.52*	1082.43±283.62*
F		8.77	5.41	18.43	281.64
p		<0.01	<0.01	<0.01	<0.01
*Note: *p<0.05 versus model group.*					

Results

Modeling results

Among 40 rats, 31 rats were successfully modeled, 5 rats failed to be modeled, and 4 rats died. Thus, 16 rats were included in the model group and 15 rats in the treatment group. Only 1 rat died from control group.

Blood glucose, insulin, glycosylated hemoglobin, triglyceride, and total cholesterol

The levels of blood glucose, triglyceride and total cholesterol in the model group were remarkably higher than those in the treatment group and control group (p<0.01), while the level of glycosylated hemoglobin was lower than that in the treatment group and the control group (p<0.01). Various indicators in the treatment group were close to the normal levels after the injection of 25-hydroxyvitamin D. (Table I).

Serum calcium, phosphorus, ALP, BGP, and TRACP

The levels of serum calcium, phosphorus, BGP and ALP in the model group were significantly lower than those in the treatment and control groups (p<0.01). There were no significant differences between the treatment group and the control group (p>0.05). The activity of TRACP in the model group was higher than those in the treatment group and the control group (p<0.01) (Table II).

Detections results via X-ray

The density of the lumbar vertebra, femur, and tibia in the model group was lower than that in the treatment group and the control group (p<0.01). There was no significant difference between the treatment group and the control group (p>0.05) (Table III).

Vertebral microstructure

The bone mineral density, trabecular thickness, trabecular number and trabecular separation degree in the model group were lower than those in the treatment group and the control group (p<0.01). The bone trabeculae presented a dense tridimensional network structure in the control group, a roughly tridimensional network structure with rarely broken structures in the treatment group, and were sparsely arranged with fractured bone trabeculae in some parts in the model group (Table IV).

Discussion

Vitamin D is necessary for glucose to stimulate insulin secretion and maintain normal glucose tolerance, which controls the normal secretion of insulin and exerts a crucial effect on maintaining bone health[6]. However, DM is a metabolic disease with a variety of complications, which can easily trigger the loss or destruction of numerous substances maintaining the patient's bone health, among which vitamin

D is one of the major lost metabolites[7]. Hence, the effect of clinically applying 25-hydroxyvitamin D to treat DM on the bone microstructure is an extremely vital subject[8].

In this study, comparisons of the differences in various indicators among T2MD rats, T2MD rats injected with 25-hydroxyvitamin D and normal rats revealed that it was feasible to treat DM with 25-hydroxyvitamin D. The levels of blood glucose, triglyceride and total cholesterol in rats of the treatment group injected with 25-hydroxyvitamin D were markedly decreased compared with those in the model group, while the level of glycosylated hemoglobin was remarkably increased, and the levels were closer to those of rats in the normal control group. Comparisons of the serum calcium, phosphorus, ALP, BGP, TRACP, the bone mineral density at the backbone parts and bone microstructure among three groups manifested that each indicator of rats in the model group was lower than those in the other two groups, with sparsely arranged bone microstructures. After treatment with 25-hydroxyvitamin D, the bone health of rats in the treatment group was better than that in the model group, indicating that 25-hydroxyvitamin D can significantly improve the bone health of rats during the treatment of T2MD. 25-hydroxyvitamin D stimulates the metabolism and absorption of calcium and phosphorus in the intestines, kidneys, and bones, and maintains the bone metabolism at the normal level[9,10]. In the bone, 25-hydroxyvitamin D promotes, mineralizes and differentiates the bone matrix, osteoid and bone cells[11]. Since high-sugar substances, large amounts of minerals and vitamins runoff in DM patients, which results in a sharp increase in glycosylation products in the advanced stage and failure of normal synthesis of ALP, BGP and other proteins in the bone. Also, the glycation product protein in the kidney stimulates monocytes and macrophages to produce interleukin-1 (IL-1) and other osteoclast factors, which increase the activity of TRACP and osteoclasts, destroy the original bone microstructure, and reduce bone density, to induce complications such as osteoporosis and rheumatism[12-14]. Insulin possesses the roles of stimulating the accumulation of amino acids, the formation of nucleotides, and the synthesis of collagen in bone cells[15]. In the absence of insulin involvement, renal tubular epithelial cells cannot normally absorb calcium and phosphorus in the blood[16]. In the treatment of DM, 25-hydroxyvitamin D can regulate the secretory function of pancreatic beta cells, causing the low expression of osteoclast differentiation factors induced by insulin and stimulating the proliferation of bone cells, which re-activate the prostrated bone cells due to lack of various proteins and improve the bone health of patients[17,18]. The increased sensitivity of peripheral insulin effectively reduces the rates of blood glucose and lipids, stimulates renal 1α-hydroxylase and maintains the synthesis of active vitamin D in the body. Naturally, 25-hydroxyvitamin D is obviously effective in the treatment of DM mainly manifested as increased blood glucose and loss of massive glucose metabolites. At the same time, it can promote the formation of bones and maintain the bone microstructure[19,20].

The rat model of T2MD was established in this experiment to study the effect of 25-hydroxyvitamin D on the bone microstructure in DM patients. Nevertheless, the short experimental time and small sample size can only indicate the changes in the bone microstructure of DM patients in the early stage. Further experiments need to be conducted so as to explore the bone microstructures of various types of DM in each stage after the treatment with 25-hydroxyvitamin D. In conclusion, it is feasible to treat rats with T2MD with 25-hydroxyvitamin D, which can maintain the integrity of bone microstructure and increase the bone health.

Authors' contributions

JG and DS were responsible for model construction. LT and BS performed ELISA. XN and WL worked on the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the ethics committee of Yantaishan Hospital.

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