Int J Clin Exp Med 2016;9(6):9425-9430
www.ijcem.com /ISSN:1940-5901/IJCEM0006174

Original Article

Effect of 1, 25-Dihydroxyvitamin D3 on triglyceride content and expressions of protein tyrosine phosphatase-1B in liver of type 2 diabetes mellitus rat

Yan Yang[1], Beibei Liu[1], Lin Gao[1], Dejing Wang[2], Qirong Chen[1], Qian Wang[1], Meihui Song[1]

[1]*Department of Endocrinology, The First Affiliated Hospital of Zunyi Medical College, Zunyi 563099, China;*
[2]*Department of Reproductive Center, The First Affiliated Hospital of Zunyi Medical College, Zunyi 563099, China*

Received January 20, 2015; Accepted June 15, 2015; Epub June 15, 2016; Published June 30, 2016

Abstract: The aims to observe the impacts and regulation mechanisms of 1, 25 hydroxy Vitamin D3 [1, 25$(OH)_2$D3] on the triglyceride contents and expression of protein tyrosine phosphatase-1B (PTP-1B) in the liver of type 2 diabetes mellitus (T2DM) rats. 120 health male Wistar Rats were randomly divided into four groups: the T2DM group; the 1, 25$(OH)_2$D3-treatmwnt group (the VitD group); the peroxisome proliferator-activated receptors a (PPAR-a) inhibitor-treatment group the VitD + PPAR-a inhibitor group) and the normal control group (the control group). The blood biochemical indicators and liver PPAR-a and PTP-1B gene and protein expression were detected in using real time reverse transcription polymerase chain reaction and Western blot, respectively. We also detected the level of triglyceride in the livers. The gene and protein expressions of PPAR-a of the T2DM group were lower than the control group ($P < 0.05$). The gene and protein expressions of PTP-1B and the TG in the T2DM group were higher than that in the control group ($P < 0.05$). Vitamin D treatment enhanced the gene and protein expressions of PPAR-a when compared with the T2DM group ($P < 0.05$). In contrast, Vitamin D suppressed PTP-1B expression in both gene and protein levels and lowered the TG content when compared with the T2DM group ($P < 0.05$). However, the effects of vitamin D on diabetes rats were reversed by PPAR-a inhibitor, especially in TG contents and PTP-1B expression. Therefore, 1, 25$(OH)_2$D3 exerted the suppression effects on TG levels and PTP-1B expression in T2DM rats model, at least in part, through PPAR-a signaling.

Keywords: 1, 25-Dihydroxyvitamin D3, protein tyrosine phosphatase-1B, triglyceride, type 2 diabetes mellitus

Introduction

Protein tyrosine phosphatase-1B (PTP-1B) was one new therapeutic target towards the treatment of type 2 diabetes mellitus (T2DM), obesity and dyslipidemia, etc. The latest studies had found that PTP-1B was closely related with T2DM and lipid metabolism [1-3]. The studies in recent years had found that the active vitamin D was closely linked with the occurrence and development of T2DM and abnormal lipid metabolism [4-10], which also played a positive role towards the glycemic control of T2DM patients, as well as improved the insulin sensitivity [11, 12]. The active vitamin D's roles of improving the hepatic steatosis and insulin resistance (IR) inside the liver were through its receptor-activated peroxisome proliferator-activated receptors a (PPAR-a) signaling pathway [13-15]. While currently, the relationships between the active vitamin D and PTP-1B, as well as the specific mechanisms through which it reduced the liver TG, were still clear, the present study observed the impacts of the active vitamin D on the gene and protein expressions of PTP-1B and the TG contents inside the T2DM rats' livers, aiming to initially explore the mechanisms of the active vitamin D in regulating the liver TG metabolism, which might provide new ideas for the prevention of hypertriglyceridemia, IR and diabetes.

Materials and methods

Animals

120 clean healthy male Wistar rats, with an average age as 6 weeks old, and the average

weight as 210.25±11.26 g, were purchased from the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences (certificate number 20020001). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Zunyi Medical College.

Animal grouping and establishment of rat T2DM model

All the rats were fed with the normal diet for the adaptability for two weeks, then randomly divided into the T2DM group, the VitD + DM group, the VitD + PPAR-a inhibitor + DM group and the normal control group (the control group), with 30 rats in each group. The T2DM group was given the high-fat high-cholesterol diet for four weeks, then after 16-hour fasting, the intraperitoneal injection of 30 mg/kg streptozotocin (STZ, 0.5% citrate buffer solution, PH 7.2) was performed towards each rat, and the blood glucose was tested from the tail venous blood 72 hours later, the criteria of successful diabetes modeling was set as the blood glucose concentration > 16.7 mmol/L, after the modeling, the T2DM group and the PPAR-a inhibitor group were intraperitoneally injected the same amount of peanut oil as the 1, 25$(OH)_2$D3 group every other day for a total of seven times. The VitD group was intraperitoneally injected 1, 25$(OH)_2$D3 (5 μg kg^{-1}) every other day for a total of seven times. Before the injection, the rat was firstly fixed onto the operating table, and the injection site was selected at the slightly left site towards the abdominal white line in the lower abdomen, the needle should pierce into the skin from the lower abdomen toward the head, when the needle reached the hypodermis, it was advanced another 3-5 ml, then pieced into the abdominal muscles with 45° angle towards the skin, when the needle penetrated the abdominal muscles, the resistance disappeared. Then, held the needle at this point stably, and withdrew the syringe, if there was no return of blood or urine, the liquid inside could be then gently injected into with a certain speed, after the injection was completed, pressed the injection site to prevent the spilling of liquid. All the animals were killed at the end of the experiment two weeks later. The enzymatic colorimetry was performed to measure the hepatic triglyceride (TG) content (the kit was purchased from the BioSino Bio-technology and Science Inc).

Real time qRT-PCR

The total hepatic RNA was extracted, and 1.5 μg total RNA was sued as the template for the reverse transcription reaction, with the total volume of reverse transcription reaction system as 20 μL. The primers of PPAR-a, PTP-1B and internal reference (β-actin) were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. as the following: PPAR-a: normal sense primer: 5'-CAGGCTATCATTACGGAGTC-3'; antisense primer: 5'-CTGGCATTTGTTTCTGTTCT-3'; PTP-1B normal sense primer: 5'-TAGTTGCGTTACACCCTTTCTTG-3'; antisense primer: 5'-TGCTGTCACCTTCACCGTTC-3'. β-acti normal sense primer: 5'-TAGTTGCGTTACACCCTTTCTTG-3'; antisense primer: 5'-TGCTGTCACCTTCACCGTTC-3'. Reaction conditions: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 58-75°C for 30 s, extension at 72°C for 1 min, 28-33 cycles, followed by the terminal extension at 72°C for 5 min. The annealing temperatures and the cycle numbers of the three genes were: PPAR-a 57°C, 30 cycles; PTP-1B 60°C, 32 cycles; β-actin 56°C, 26 cycles. To correct the error, this study used the housekeeping gene β-actin as the internal control, the average copy numbers of the sample's target gene were divided by the average copy numbers of the reference gene inside the sample, thus the relative content of the target gene could be obtained. The copy numbers of the template in the sample could be calculated by the SDS software, which used the Ct value obtained and put it into the standard curve.

Western blot

The liver tissues of each group were collected and performed the quantification by the BCA method. 30 μg protein sample was taken for the 12% PAGE electrophoresis, then transferred onto a PVDF membrane, then the primary antibodies of PPAR-a, PTP-1B (R&D, USA) (with the dilution as 1:2000) and the secondary antibodies (R&D, USA) (with the dilution as 1:1000) were taken for the alkaline phosphatase staining and exposure.

Table 1. Levels of weight and glucose in different groups

Group	n	Weight (g)	Glucose (mmol/L)
CON	25	285.2±11.57	5.38±0.71
T2DM	25	381±9.3[a]	7.39±1.67[a]
VitD	25	378.6±8.6[a,b]	6.98±1.47[a,b]
VitD + PPAR-a inhibitor	25	383.6±9.1[a,b]	7.12±1.52[a,b]

Compare with the control group, [a]P < 0.05; Compare with the T2DM group, [b]P < 0.05.

Table 2. Levels of TG, TC, HDL-C and LDL-C in different groups

Group (mmol/L)	n	TG	TC	HDL-C	LDL-C
CON	25	0.41±0.07	1.33±0.16	0.35±0.05	0.37±0.07
T2DM	25	0.72±0.25[a]	5.42±0.72[a]	0.35±0.06	1.13±0.41[a]
VitD	25	0.43±0.11[a,b]	5.31±0.61[a]	0.36±0.07	1.09±0.42[a]
VitD + PPAR-a inhibitor	25	0.73±0.31[a]	5.28±0.65[a]	0.34±0.07	1.12±0.50[a]

Compare with CON group, [a]P < 0.05, Compare between different concentrations of group, [b]P < 0.05.

Statistical analysis

The SPSS16.0 statistical package was used for the statistical analysis, the results were expressed as mean ± standard deviation ($\overline{x}$±s), the normally distributed data were performed the random block design for the data's analysis of variance and pairwise comparison, the gene and protein expression differences of PPAR-a and PTP-1B, as well as the TG content of each group, were compared, with P < 0.05 considered as the statistical significance.

Results

Body weight, blood glucose and lipid level

Compared with the control group, the body weights of the T2DM group, the VitD group and the PPAR-a inhibitor group were significantly increased, and the blood glucose levels were significantly increased (P < 0.05), the TG, TC and LDL-C levels were significantly higher (P < 0.05), while the HDL-C levels did not change significantly (P > 0.05). Compared with the T2DM group, the body weights and blood glucose levels of the VitD group and the PPAR-a inhibitor group were not significantly different (P > 0.05), the TG level of the VitD group was significantly lower than the T2DM group (P < 0.05), while the TC, LDL-C and HDL-C levels had no significant changes between the 2 groups (P > 0.05); compared with the T2DM group, the TG, TC, LDL-C and HDL-C levels of the PPAR-a inhibitor group had no significant changes (P > 0.05). Compared with the PPAR-a inhibitor group, the body weight and blood glucose of the VitD group were not significantly different (P > 0.05); the serum TG level was significantly lower (P < 0.05), while the TC, LDL-C and HDL-C levels had no significant changes (P > 0.05) (**Tables 1**, **2**).

Expression of PPAR-a and PTP-1B

The gene and protein expressions of PPAR-a of the T2DM group were significantly reduced than the control group (P < 0.05), while those of PTP-1B and the TG content were significantly higher than the control group (P < 0.05). The gene and protein expressions of PPAR-a of the VitD group were significantly increased than the T2DM group (P < 0.05), while those of PTP-1B were significantly inhibited (P < 0.05), and the TG content was significantly reduced than the T2DM group (P < 0.05). The gene and protein expressions of PPAR-a and PTP-1B of the VitD group had no significant difference than the control group (P > 0.05), and the comparison of TG contents between the 2 groups had no significant difference (P > 0.05). The gene and protein expressions of PPAR-a of the PPAR-a inhibitor group were significantly reduced than the control group (P < 0.05), while those of PTP-1B were significantly increased (P < 0.05), and the TG content was significantly increased than the control group (P < 0.05). The gene and protein expressions of PPAR-a of the PPAR-a inhibitor group were significantly lower than the T2DM group (P < 0.05), while those of PTP-1B and the TG content had no significant differences when compared with the T2DM group (P < 0.05). The gene and protein expressions of PPAR-a of the PPAR-a inhibitor group was significantly lower than the VitD group (P < 0.05), while those of PTP-1B and the TG content were significantly increased than the VitD group (P < 0.05) (**Table 3**; **Figures 1**, **2**).

Discussion

The liver was the important organ for the triglyceride metabolism, the hypertriglyceridemia

Table 3. The comparison of PPAR-α, PTP-1B gene expression and triglyceride levels of different group ($\bar{x}$±s)

Group	CON	T2DM	VitD	VitD + PPAR-αinhibitor
N	25	25	25	25
PPAR-αmRNA	6.87±1.87	2.15±0.27[a,b]	6.75±1.59	0.89±0.12[a,b]
PTP-1B mRNA	2.32±0.28	7.27±2.14[a,b]	2.38±0.32	7.26±2.15[a,b]
TG (mg/g)	208.14±26.37	434.13±37.35[a,b]	210.±26.34	433.23±37.25[a,b]

Compare with control group, [a]P < 0.05; Compare between different concentrations of group, [b]P < 0.05.

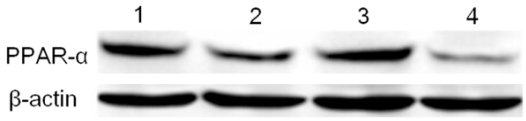

Figure 1. Comparison of PPAR-a protein expression in liver between various group. Lane 1: control group; Lane 2: T2DM group; Lane 3: VitD group; Lane 4: VitD + PPAR-a inhibitor.

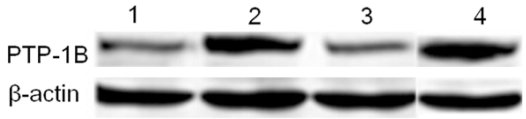

Figure 2. Comparison of PTP-1B protein expression in liver between various group. Lane 1: control group; Lane 2: T2DM group; Lane 3: VitD group; Lane 4: VitD + PPAR-a inhibitor.

was the important pathophysiological basis and characteristics towards the occurrence and development of diabetes [16]. The recent studies had found that the vitamin D not only was involved in such diseases as the skeletal system diseases, cancer, autoimmune diseases, cardiovascular and respiratory diseases, but also participated the occurrence and development of obesity and diabetes, etc [17]. PTP-1B could be used as a target towards such disease-related treatments as T2DM, obesity and IR, the inhibition of PTP-1B expression inside the liver could increase the insulin sensitivity, reduce the blood sugar level of the diabetic rats, as well as regulate the lipid metabolism [18, 19]. The foreign latest study found that the plasma 25 (OH) D was negatively correlated with IR, the increasing of plasma 25 (OH) D levels in the T2DM patients could significantly improve IR, the vitamin D was closely related to the insulin secretion and insulin sensitivity, the vitamin D deficiency was closely associated with the occurrence of diabetes, and the supplement of vitamin D could prevent T2DM [20, 21]. The foreign study also found that the plasma 25 (OH) D level was closely related with the hyperlipidemia [22], but how was the relationships between the vitamin D and PTP-1B in the treatment of T2DM, IR and hypertriglyceridemia was not reported domestically and abroad, so we intraperitoneally injected the active vitamin D into the T2DM rats, then detected the gene and protein expressions of PTP-1B and TG levels inside the livers, aiming to preliminarily study the possible mechanisms of active vitamin D in regulating the hepatic TG metabolism of T2DM rats.

This study found that: ① the gene and protein expressions of PPAR-a of the T2DM group were significantly reduced than the control group (P < 0.05), while those of PTP-1B and the TG content were significantly higher than the control group (P < 0.05); ② the gene and protein expressions of PPAR-a of the VitD group were significantly increased than the T2DM group (P < 0.05), while those of PTP-1B were significantly inhibited (P < 0.05), and the TG content was significantly reduced than the T2DM group (P < 0.05); ③ the gene and protein expressions of PPAR-a and PTP-1B of the VitD group had no significant difference than the control group (P > 0.05), and the comparison of TG contents between the 2 groups had no significant difference (P > 0.05); ④ the gene and protein expressions of PPAR-a of the PPAR-a inhibitor group were significantly reduced than the control group (P < 0.05), while those of PTP-1B were significantly increased (P < 0.05), and the TG content was significantly increased than the control group (P < 0.05); ⑤ the gene and protein expressions of PPAR-a of the PPAR-a inhibitor group were significantly lower than the T2DM group (P < 0.05), while those of PTP-1B and the TG content had no significant differences when compared with the T2DM group (P < 0.05); ⑥ the gene and protein expressions of PPAR-a of the PPAR-a inhibitor group was significantly lower than the VitD group (P < 0.05), while those of PTP-1B and the TG content were significantly increased than the VitD group (P <

0.05). The results of this study showed that the active vitamin D was closely related to the hepatic PTP-1B expression and TG level, 1, $25(OH)_2D3$ could reduce the hepatic TG content, which might be achieved through inhibiting the expression of PTP-1B.

In summary, the hepatic PPAR-a expression of T2DM rats were reduced, while the PTP-1B expression was increased, and the TG level was increased, resulting in the hypertriglyceridemia, furthermore, the hypertriglyceridemia would increase the TG deposition inside the liver, thus increasing the expression of PTP-1B, which formed a vicious circle. Increasing the plasma 1, $25(OH)_2D3$ level could raise the hepatic PPAR-a expression, inhibit the PTP-1B expression, thereby reducing the TG level and improving IR, while the exact mechanism still remained the further exploration.

Acknowledgements

National Natural Science Foundation of China (81460168); Guizhou provincial Science and Technology Fund Project {Number: Qian Ke He J LKZ[2013]19}; The doctoral scientific research starting up fund of Zunyi Medical College ([2012]F-574).

Disclosure of conflict of interest

None.

Address correspondence to: Lin Gao, Department of Endocrinology, The First Affiliated Hospital of Zunyi Medical College, No. 201 Dalian Road Huichuan District, Zunyi 563099, China. Tel: +86 13595286671; Fax: +86 0852 8609200; E-mail: lingaodoc@126.com

References

[1] Wang CD, Teng BS, He YM, Wu JS, Pan D, Pan LF, Zhang D, Fan ZH, Yang HJ and Zhou P. Effect of a novel proteoglycan PTP1B inhibitor from Ganoderma lucidum on the amelioration of hyperglycaemia and dyslipidaemia in db/db mice. Br J Nutr 2012; 108: 2014-2025.

[2] Kubo M, Fukui M, Ito Y, Kitao T, Shirahase H, Hinoi E and Yoneda Y. Insulin sensitization by a novel partial peroxisome proliferator-activated receptor γ agonist with protein tyrosine phosphatase 1B inhibitory activity in experimental osteoporotic rats. J Pharmacol Sci 2014; 124: 276-285.

[3] Davis JA, Sharma S, Mittra S, Sujatha S, Kanaujia A, Shukla G, Katiyar C, Lakshmi BS, Bansal VS and Bhatnagar PK. Antihyperglycemic effect of Annona squamosa hexane extract in type 2 diabetes animal model: PTP1B inhibition, a possible mechanism of action? Indian J Pharmacol 2012; 44: 326-332.

[4] Eftekhari MH, Akbarzadeh M, Dabbaghmanesh MH and Hassanzadeh J. The effect of calcitriol on lipid profile and oxidative stress in hyperlipidemic patients with type 2 diabetes mellitus. ARYA Atheroscler 2014; 10: 82-88.

[5] Hirani V, Cumming RG, Le Couteur DG, Naganathan V, Blyth F, Handelsman DJ, Waite LM and Seibel MJ. Low levels of 25-hydroxy vitamin d and active 1, 25-dihydroxyvitamin d independently associated with type 2 diabetes mellitus in older Australian men: the concord health and ageing in men project. J Am Geriatr Soc 2014; 62: 1741-1747.

[6] Kampmann U, Mosekilde L, Juhl C, Moller N, Christensen B, Rejnmark L, Wamberg L and Orskov L. Effects of 12 weeks high dose vitamin D3 treatment on insulin sensitivity, beta cell function, and metabolic markers in patients with type 2 diabetes and vitamin D insufficiency-a double-blind, randomized, placebo-controlled trial. Metabolism 2014; 63: 1115-1124.

[7] Liu Z, Liu L, Chen X, He W and Yu X. Associations study of vitamin D receptor gene polymorphisms with diabetic microvascular complications: a meta-analysis. Gene 2014; 546: 6-10.

[8] Al-Daghri NM, Al-Attas OS, Alkharfy KM, Khan N, Mohammed AK, Vinodson B, Ansari MG, Alenad A and Alokail MS. Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. Gene 2014; 542: 129-133.

[9] Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, Pittas AG, Boland R, Ferrucci L and Bikle DD. Vitamin D: beyond bone. Ann N Y Acad Sci 2013; 1287: 45-58.

[10] Li L, Wu B, Liu JY and Yang LB. Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. Arch Med Res 2013; 44: 235-241.

[11] Jehle S, Lardi A, Felix B, Hulter HN, Stettler C and Krapf R. Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: a randomised, placebo-controlled, prospective pilot study. Swiss Med Wkly 2014; 144: w13942.

[12] Gandhe MB and Jain K. Gandhe SM/Evaluation of 25(OH) Vitamin D3 with reference to magnesium status and insulin resistance in T2DM. J Clin Diagn Res 2013; 7: 2438-2441.

[13] Yin Y, Yu Z, Xia M, Luo X, Lu X and Ling W. Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. Eur J Clin Invest 2012; 42: 1189-1196.
[14] Lebrun V, Molendi-Coste O, Lanthier N, Sempoux C, Cani PD, van Rooijen N, Stärkel P, Horsmans Y, Leclercq IA. Impact of PPAR-α induction on glucose homoeostasis in alcohol-fed mice. Clin Sci (Lond) 2013; 125: 501-511.
[15] Zhao XQ, Quan L, Du GL, Xiao S, Lü WK and Zhu J. [Protective effects and mechanisms of 1, 25(OH)2D3 on diabetes-induced liver injury in a streptozotocin-diabetic rat model]. Zhonghua Gan Zang Bing Za Zhi 2013; 21: 778-780.
[16] Feher M, Greener M and Munro N. Persistent hypertriglyceridemia in statin-treated patients with type 2 diabetes mellitus. Diabetes Metab Syndr Obes 2013; 6: 11-15.
[17] Roxana VR, Laura GL, Elina MC and Donají BA. Vitamins And Type 2 Diabetes Mellitus. Endocr Metab Immune Disord Drug Targets 2015; 15: 54-63.
[18] Goldstein BJ. Protein-tyrosine phosphatase 1B (PTP1B): a novel therapeutic target for type 2 diabetes mellitus, obesity and related states of insulin resistance. Curr Drug Targets Immune Endocr Metabol Disord 2001; 1: 265-275.
[19] Tiganis T. PTP1B and TCPTP-nonredundant phosphatases in insulin signaling and glucose homeostasis. FEBS J 2013; 280: 445-458.
[20] Wang G, Li Y, Li L, Yu F, Cui L, Ba Y, Li W and Wang C. Association of the vitamin D binding protein polymorphisms with the risk of type 2 diabetes mellitus: a meta-analysis. BMJ Open 2014; 4: e005617.
[21] Xuan Y, Zhao HY and Liu JM. Vitamin D and type 2 diabetes mellitus (D2). J Diabetes 2013; 5: 261-267.
[22] Jorde R and Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. Prog Lipid Res 2011; 50: 303-312.