European Review for Medical and Pharmacological Sciences 2016; 20: 2916-2919

Role of vitamin D_3 in regulation of interleukin-6 and osteopontin expression in liver of diabetic mice

D. LABUDZYNSKYI, I. SHYMANSKYY, M. VELIKY

Laboratory of Medical Biochemistry, O.V. Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv

Abstract. – OBJECTIVE: To study the link between hepatic interleukin-6 (IL-6) and osteopontin (OPN) gene expression and vitamin D_3 status associated with type 1 diabetes in mice; and to evaluate the effects of vitamin D_3 treatment (800 IU/kg of body weight for 6 weeks) on diabetes-induced impairments.

MATERIALS AND METHODS: mRNA levels of IL-6 and OPN were measured by quantitative RT-PCR. Blood serum $25OHD_3$ was assayed by ELISA.

RESULTS: It was shown that induction of IL-6 in diabetic liver is accompanied by increased expression of OPN. Changes in OPN and IL-6 RNA levels correlated with a lack of $25OHD_3$ in serum. Vitamin D_3 treatment restored $25OHD_3$ that led to a substantial reduction of OPN and IL-6 mRNA levels.

CONCLUSIONS: Diabetes-induced vitamin D_3 deficiency was associated with increased hepatic levels of IL-6 and OPN mRNA and these changes were countered by vitamin D_3 administration.

Key Words:
Type 1 diabetes, Liver, Inflammation, Vitamin D_3, Interleukin-6, Osteopontin.

Introduction

Type 1 diabetes mellitus (DM1) is a multifactorial autoimmune disease characterized by a genetic predisposition and progressive loss of insulin-producing pancreatic beta cells[1]. The development of chronic hyperglycemia together with oxidative stress and immune disorders are implicated in the pathogenesis of common and devastating complications of DM1, including liver disease[2].

Inflammation is one of the mechanisms of liver injury in diabetes[3]. The rise in proinflammatory cytokines favours diabetes-related glucose toxicity, leading to mitochondrial dysfunction, oxidative stress and hepatocellular death. Growing body of epidemiological, genetic and experimental evidence demonstrated a significant role of interleukin-6 (IL-6) in the pathogenesis of inflammation, insulin resistance, diabetes and its complications[4,5].

More recently it was shown, that osteopontin (OPN), a matrix extracellular glyco-phosphoprotein and a known regulator of bone formation/resorption, also plays a role in immune system signaling and inflammatory process. In particular, one of the biochemical effects of OPN interaction with the integrin receptors $\alpha v\beta 3$ is to induce IL-6 expression[6]. It is known that OPN gene promoter contains specific binding areas, including those for glucocorticoid and vitamin D_3 receptors (VDR). The presence of such gene promoter loci can open prospects for regulation of OPN-dependent genes by these biologically active compounds.

Observational studies have demonstrated association between decreased vitamin D_3 level and increased susceptibility to DM. In addition to the involvement of vitamin D_3 in the regulation of mineral metabolism, mineralization and remodeling of bone tissue, its normally active form – $1,25(OH)_2D_3$ – was shown to exhibit immunomodulatory, anti-inflammatory and antiproliferative effects that may have potential in the prevention of autoimmune diseases[7,8]. The vast majority of molecular effects that provide cytoprotective properties of vitamin D_3 are realized through VDR and genomic regulation that, in whole, corresponds to the mechanism of action of steroid hormones.

The study was designed to assess how the impairment of IL-6 and OPN gene expression in diabetic liver is associated with the bioavailability

Corresponding Author: Ihor Shymanskyy, Ph.D; e-mail: ishymansk@inbox.ru

of vitamin D_3 and to establish whether DM1-induced changes are regulated by vitamin D_3 administration.

Materials and Methods

Experimental Animals

DM1 was induced in male C56Bl/J6 mice (21 ± 3 g) by intraperitoneal injection of streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) at dose 150 mg/kg of body weight. After development of a stable hyperglycemia (2 weeks) the animals were treated daily with or without an aqueous suspension of vitamin D_3 (DSM, Heerlen, Netherlands) for 6 weeks at dose 800 IU/kg of body weight (per os). All animals received care in accordance to the guidelines approved by institutional Committee for Care and Use of Laboratory Animals in Research.

Immunoenzyme Assay

Vitamin D_3 bioavailability was estimated by the level of blood serum $25OHD_3$, which was determined by immunoenzyme technique (ELISA kit, Immunodiagnostic Systems Ltd., USA) according to the manufacturer's instructions.

Quantitative RT-PCR Analysis

Total RNA was extracted from the tissues using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA). The cDNA was synthesized from 1 µg of total RNA using random primers and Moloney murine leukemia virus reverse transcriptase (both from Life Technologies, Carlsbad, CA, USA) as previously described[9]. The primer sequences used for IL-6 were forward, 5′-AGAAGTCGGAGGCTTAATTACACAT-3′ and reverse, 5′-TTGCCATTGCACAACTCTTTTC-3′. The primer sequences used for OPN were forward, 5'-CTTTCACTCCAATCGTCCCTAC-3' and reverse, 5′-GCTCTCTTTGGAATGCTCAAGT-3′. Quantitative RT-PCR analysis was performed using the Mx3005P Real-Time PCR System (Stratagene, La Jolla, CA, USA). For each condition, expression was quantified in triplicate, and 18S rRNA was used as the endogenous control in the comparative cycle threshold (C_T) method. Data were expressed as relative expression ratio.

Statistical Analysis

The data were expressed as mean ± SEM deviation of at least three independent experiments. Statistical differences between the various groups were compared by using Student's *t*-test and one-way ANOVA. A value of $p < 0.05$ was considered statistically significant.

Results

It was shown that mean glucose level in mice with experimental DM1 reached 22.1 ± 4.4 mmol/L compared with 5.7 ± 0.5 mmol/L in control group (Table I). Chronic hyperglycemia was associated with a significant (2.9-fold) increase in IL-6 mRNA expression in liver tissue compared to control animals ($p < 0.05$) that can both reflect and contribute to inflammation and liver injury caused by DM1, Figure 1 (A). Overexpression of IL-6 mRNA correlated with 1.8-fold enhancement of osteopontin mRNA expression in liver compared with controls (Figure 1 (B); $p < 0.05$). Diabetes was accompanied by a severe deficiency of vitamin D_3 as is evident from 2.2-fold decrease in blood serum level of $25OHD_3$ in diabetic mice compared with controls animals (Table I; $p < 0.05$). A shift of serum $25OHD_3$ towards control values in diabetic mice was observed after chronic administration of vitamin D_3 ($p < 0.05$). On the background of $25OHD_3$ restoration in blood serum, it was revealed a 1.4-fold lowering of hepatic OPN mRNA expression in vitamin D_3 treated animals compared with DM1 ($p < 0.05$). The level of IL-6 mRNA was also shown to be significantly decreased (1.6-fold) in liver tissue compared with the values of diabetic group ($p < 0.05$).

Table I. Whole blood glucose and blood serum $25OHD_3$, M ± m, n = 8.

Experimental groups	$25OHD_3$ concentration, nmol/L	Glucose concentration, mmol/L
Control	81.7 ± 4.13	5.7 ± 0.5
Diabetes	37.9 ± 2.12*	22.1 ± 4.4*
Diabetes + D_3	77.3 ± 5.48$^{\#}$	15.2 ± 3.3

*$p < 0.05$ *vs*. control; $^{\#}p < 0.05$ *vs*. diabetes.

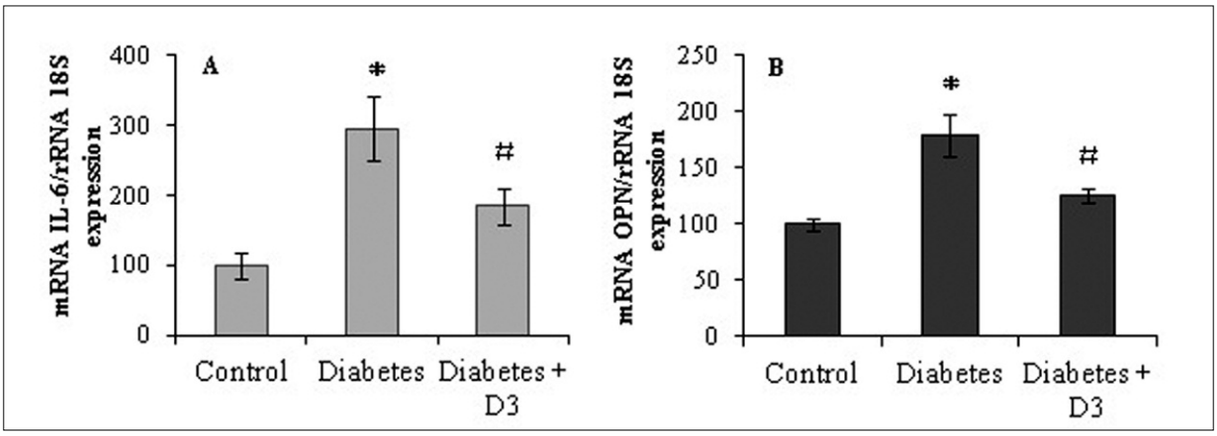

Figure 1. The levels of IL-6 ***(A)*** and OPN ***(B)*** mRNA in diabetic mice with or without vitamin D_3 administration. Data are presented as mean ± SEM of triplicate measurements (n = 8); $^{*}p < 0.05$ *vs*. control, $^{\#}p < 0.05$ *vs*. diabetes

Discussion

Emerging evidence suggests that proinflammatory cytokines contribute to the development of hepatic disease in both type 1 and 2 DM[3,4]. OPN-mediated gene expression of interleukin-6 has recently attracted attention in inflammation research field[6]. The data reported here demonstrate a simultaneous increase in gene expression of IL-6 and OPN in diabetic liver that can promote hepatic inflammation. DM1 may induce IL-6 expression and liver damage in a way that resembles the mechanism described previously in the study on primary culture of chondrocytes obtained from patients with osteoarthritis[10]. It was shown that incubation of chondrocytes with recombinant OPN results in dose-dependent IL-6 mRNA overexpression. OPN action in liver is probably related to its binding to integrin receptor $\alpha v\beta 3$ located on hepatic macrophages and other leukocytes, followed by stimulation of proinflammatory signaling transduction in these cells and subsequent expression of proinflammatory cytokines, including IL-6[6].

In accordance with previous studies[11], we further confirmed that vitamin D_3 exerts anti-inflammatory effects in experimental DM1. Our results have shown the effectiveness of vitamin D_3 in reducing RNA levels of the proinflammatory mediators, IL-6 and OPN, in liver tissue of diabetic mice. Moreover, as repletion of serum $25OHD_3$ down-regulated OPN and IL-6 expression, we can speculate that vitamin D_3 deficiency may facilitate the activity of these proinflammatory factors.

It can be suggested that vitamin D_3 action on IL-6 mRNA expression is mediated through its regulatory effect on OPN gene. Several studies[6,12] have reported the involvement of $1,25(OH)_2D_3$ in the transcriptional regulation of various osteokines, including OPN, in different cell types of bone tissue. In addition to the studies on bone tissue, there are reliable data concerning regulatory OPN-mediated influence of vitamin D_3 on metabolic and signaling processes in other tissues that might be promising in the developing of new therapeutic approaches for the treatment of a variety of chronic diseases[13]. The underlying mechanism of such regulation involves the binding of $1,25(OH)_2D_3$ to VDR receptor, followed by translocation of the active $1,25(OH)_2D_3$/VDR complex to the nucleus. Within the nucleus, the complex interacts with VDR-binding locus of OPN gene promoter, providing regulatory impact on the functioning of this gene[14].

In summary, the beneficial effects of vitamin D_3 on livers of diabetic mice indicate the importance of vitamin D_3 sufficiency in the down-regulation of IL-6 expression, which is most likely OPN-mediated, with a consequent inhibition of inflammation involved in DM1-induced liver injury.

Conclusions

Vitamin D_3 deficiency and liver damage occurring in DM1 are linked to hepatic inflammation, at least in part, due to interleukin-6 and osteopontin overexpression. Administration of vitamin D_3 causes normalization of serum $25OHD_3$ level, as a marker of optimal vitamin D_3 availability,

and down-regulates gene expression of both proinflammatory factors. Thus, our results further confirm a significant role of vitamin D_3 in the regulation of liver inflammation related to type 1 diabetes.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Pugliese A. Advances in the etiology and mechanisms of type 1 diabetes. Discov Med 2014; 8: 141-150.
2) Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010; 107: 1058-1070.
3) Ban CR, Twigg SM. Fibrosis in diabetes complications: Pathogenic mechanisms and circulating and urinary markers. Vasc Health Risk Manag 2008; 4: 575-596.
4) Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006; 17: 4-12.
5) Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes 2005; 54: 114-124.
6) Kahles F, Findeisen HM, Bruemmer D. Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes. Mol Metab 2014; 3: 384-393.
7) Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10: 482-496.
8) Labudzynskyi DO, Shymanskyy I, Riasnyi VM, Veliky MM. Vitamin D_3 availability and functional activity of peripheral blood phagocytes in experimental type 1 diabetes. Ukr Biochem J 2014; 86: 107-118.
9) Marcotorchino J, Romier B, Gouranton E, Riollet C, Gleize B, Malezet-Desmoulins C, Landrier JF. Lycopene attenuates LPS-induced TNF-alpha secretion in macrophages and inflammatory markers in adipocytes exposed to macrophage-conditioned media. Mol Nutr Food Res 2012; 56: 725-732.
10) Yang Y, Gao SG, Zhang FJ, Luo W, Xue JX, Lei GH. Effects of osteopontin on the expression of IL-6 and IL-8 inflammatory factors in human knee osteoarthritis chondrocytes. Eur Rev Med Pharmacol Sci 2014; 18: 3580-3586.
11) George N, Kumar TP, Antony S, Jayanarayanan S, Paulose CS. Effect of vitamin D_3 in reducing metabolic and oxidative stress in the liver of streptozotocin-induced diabetic rats. Br J Nutr 2012; 108: 1410-1418.
12) Staal A, Van Wijnen AJ, Desai RK, Pols HA, Birkenhäger JC, Deluca HF, Denhardt DT, Stein JL, Van Leeuwen JP, Stein GS, Lian JB. Antagonistic effects of transforming growth factor-beta on vitamin D_3 enhancement of osteocalcin and osteopontin transcription: reduced interactions of vitamin D receptor/retinoid X receptor complexes with vitamin E response elements. Endocrinology 1996; 137: 2001-2011.
13) Lau WL, Leaf EM, Hu MC, Takeno MM, Kuro-o M, Moe OW, Giachelli CM. Vitamin D receptor agonists increase klotho and osteopontin while decreasing aortic calcification in mice with chronic kidney disease fed a high phosphate diet. Kidney Int 2012; 82: 1261-1270.
14) Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α,25(OH)2vitamin D_3: genomic and non-genomic mechanisms. Best Pract Res Clin Endocrinol Metab 2011; 25: 543-559.