Derangements of vitamin D-endocrine system and NF-κB-dependent signaling are implicated in diabetes-associated osteoporosis: effect of chronic vitamin D_3 treatment

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Introduction

Background: Secondary osteoporosis is closely related to type 1 diabetes mellitus (T1D), but the underlying mechanisms are not fully defined. Diabetic-induced proinflammatory processes and failures in the D-endocrine system may have implication for the abnormal transactivation of the nuclear factor κB (NF-κB), leading to an imbalance between the formation of bone and its resorption.

Aims: The study was carried out to explore the role of NF-κB activation in a relationship with disturbances of D-endocrine system and development of secondary osteoporosis associated with experimental DM1 and to assess the potential of vitamin D_3 in correcting T1D-induced changes.

Material and methods

- Diabetes was induced in male Wistar rats by single intraperitoneal injection of streptozotocin (STZ) at dose 55 mg/kg of b.w.
- After two weeks of T1D development, all animals were divided into 4 groups: 1- control; 2- diabetes; 3- diabetes treated with 100 IU of vitamin D_3 orally during 30 days; 4- diabetes treated with selective NF-κB inhibitor BAY 11-7082 (1 mg/kg b.w., i.p for 10 days). The levels of calcium (Ca^{2+}) in blood serum and bone tissue (in ash) were determined spectrophotometrically.
- The activity of the alkaline phosphatase (AP) in serum was detected using p-nitrophenyl phosphate as a substrate.
- Vitamin D bioavailability in the organism of the experimental animals was determined by the measurement of calcidiol (25OHD) level in blood serum using the competitive immunoenzyme analysis (ELISA).
- The protein levels of total NF-κB/p65 and NF-κB/p-p65 (p65 subunit phosphorylated at Ser 311), VDR and 25-hydroxy vitamin D 1α-hydroxylase (CYP27B1) were assayed by Western blot analysis.
- Changes in the mRNA levels of vitamin D receptor and 1α-hydroxylase were measured by Real-Time quantitative PCR.

Results and discussion

T1D was associated with osteoporosis development as was evident by decreased Ca^{2+} in serum and bone tissue and increased alkaline phosphatase activity in serum. Diabetes also led to 25OHD deficiency and lowered VDR expression in bone tissue. The level of CYP27B1 protein was downregulated, while CYP27B1 mRNA expression was elevated. T1D induced an increase in total and phosphorylated at Ser311 NF-κB/p65. BAY 11-7082 treatment resulted in partial normalization of mineral metabolism, 25OHD and CYP27B1 levels. It also blocked NF-κB/p65 phosphorylation in bone tissue of T1D animals but increased the level of total NF-κB. Vitamin D_3 treatment reversed mRNA and protein levels of VDR to control values. CYP27B1 protein level rose on the background of diminished CYP27B1 mRNA. Significant downregulation of both total and phosphorylated NF-κB/p65 was achieved after vitamin D_3 treatment.

Table 1: Glucose, 25OHD, Alkaline phosphatase and Ca^{2+} level in blood, Ca^{2+} level in bone tissue of experimental STZ T1D rats and after vitamin D_3 or BAY 11-7082 treatment. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

	Control	Diabetes	Diabetes + Vitamin D_3	Diabetes + BAY 11-7082
Glucose, mmol/L	4.9±0.2	26.8±2.9*	21.7±2.5	22.9±1.3
25(OH)D, nmol/L	97.5±5.2	50.2±3.0*	71.0±3.1#	51.6±1.7
Ca^{2+} in blood, mmol/L	2.16±0.1	1.97±0.07*	2.14±0.1#	2.02±0.05#
AP in blood, μmol/min*L	251.2±10	383±4.5*	299±5#	338±3#
Ca^{2+} in bone tissue, mmol/L	31.7±0.5	29.4±1.1*	31.2±0.6#	30.9±0.5#

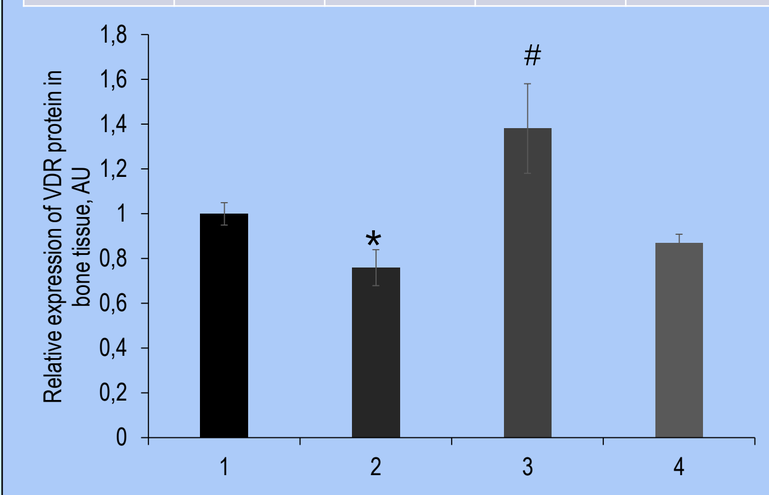

Figure 1: VDR protein level in bone tissue. Densitometric analysis of immunospecific bands. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; 4) diabetes+ 1 mg/kg b.w. of BAY 11-7082 during 10 days. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

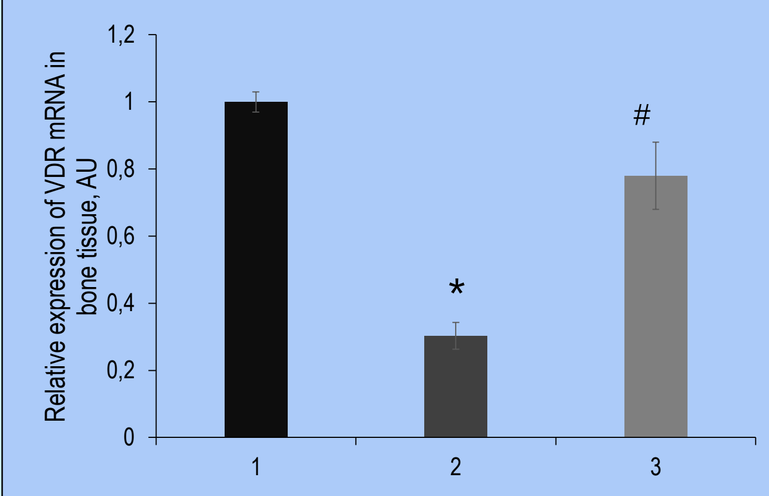

Figure 2: VDR mRNA level in bone tissue. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days;. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

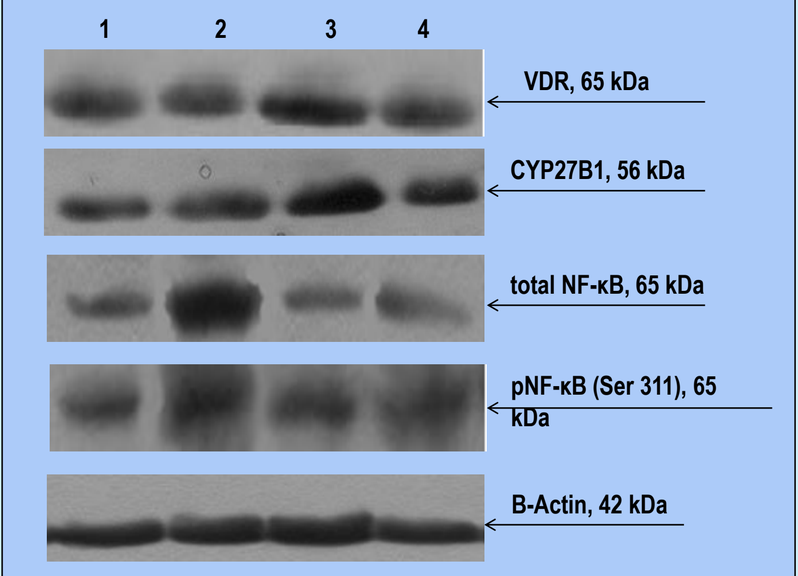

Figure 3: Representative immunoblotograms of VDR, CYP27B1, total NF-κB and pNF-κB (Ser 311) protein expression in bone tissue. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; All measurements were done in triplicate.

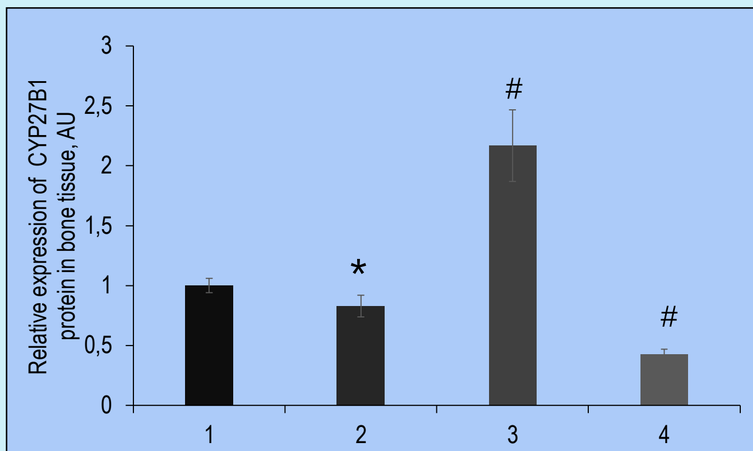

Figure 4: CYP27B1 protein level in bone tissue. Densitometric analysis of immunospecific bands. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; 4) diabetes+ 1 mg/kg b.w. of BAY 11-7082 during 10 days. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0/05 significance in comparison with diabetes group.

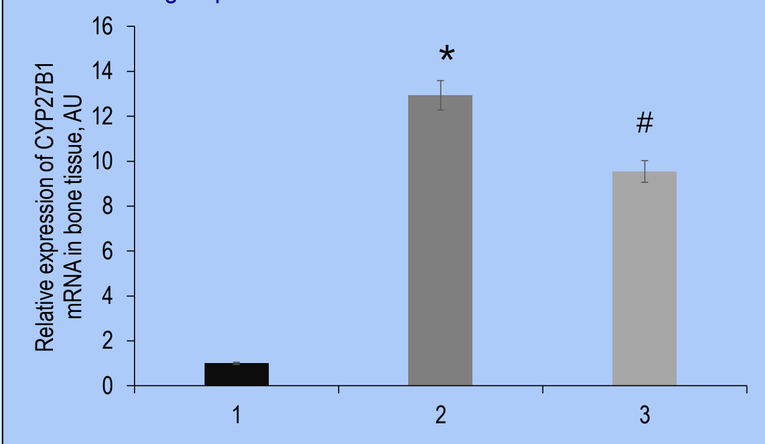

Figure 5: CYP27B1 mRNA level in bone tissue. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

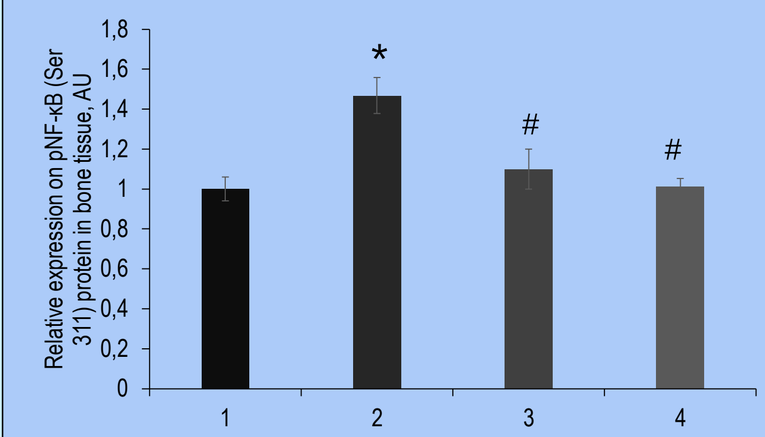

Figure 6: pNF-κB (Ser 311) protein level in bone tissue. Densitometric analysis of immunospecific bands. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; 4) diabetes+ 1 mg/kg b.w. of BAY 11-7082 during 10 days. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

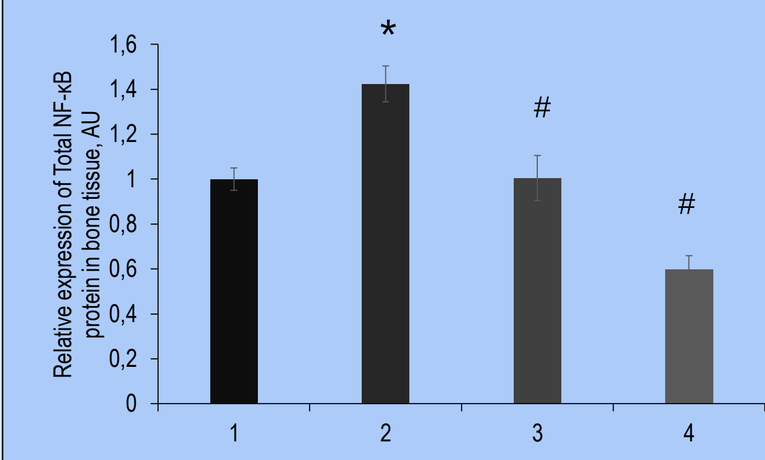

Figure 7: Total NF-κB protein level in bone tissue. Densitometric analysis of immunospecific bands. 1) control; 2) diabetes; 3) diabetes + 100 IU of vitamin D_3 during 30 days; 4) diabetes+ 1 mg/kg b.w. of BAY 11-7082 during 10 days. M±n, n=5 *p≤0.05 significance in comparison with control group. #p≤0.05 significance in comparison with diabetes group.

Conclusion

Selective NF-κB inhibition and vitamin D_3 treatment partially reversed T1D-associated abnormalities of bone remodeling, indicating the importance of vitamin D-deficiency and NF-κB transcriptional activation in elevated bone tissue resorption.