The effect of vitamin D_3 supplementation on $P2X_7$ receptor function and expression in early chronic kidney disease

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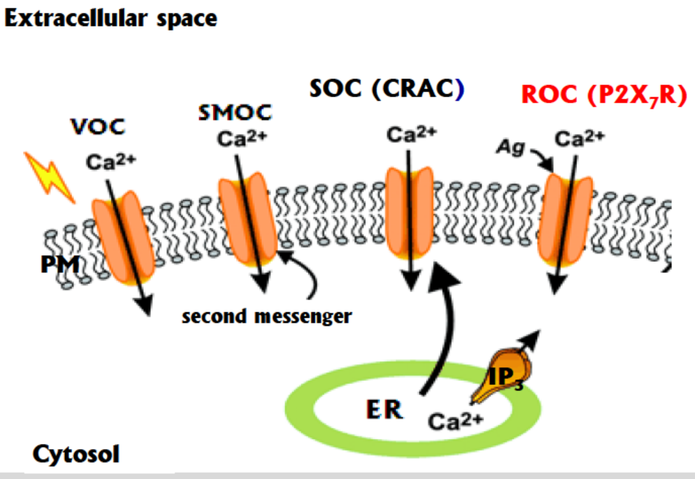
INTRODUCTION

Chronic low - grade inflammation is common in chronic kidney disease (CKD) patients. The $P2X_7$ receptor ($P2X_7R$) is increasingly recognized as an important cell surface regulator of several key inflammatory molecules. $P2X_7R$ activation by extracellular ATP results in opening the cation channel followed by forming a non-specific pore. Channel opening induces Na^+ and Ca^{2+} influx and K^+ efflux leading to plasma membrane depolarization, increase of intracellular Ca^{2+} level and activation of Ca^{2+} signalling cascades. This results in a variety of biological responses, mainly related to inflammation, cell proliferation and tissue damage.

CALCIUM ENTRY ACROSS PLASMA MEMBRANE



ROC – receptor operated channel, VOC – voltage operated channel, SOC – store operated channel, SMOC - second messenger operated channel, CRAC – calcium release activated calcium channel, PM – plasma membrane

AIM OF THE STUDY

The aim of the study was to examine the effect of vitamin D_3 supplementation on $P2X_7R$ function and expression in peripheral blood mononuclear cells (PBMCs) of patients in early stages of CKD.

SUBJECTS AND METHODS

The study involved 20 healthy volunteers and 16 non-diabetic patients with stage 2-3 CKD. CKD patients were supplemented with cholecalciferol 7 000 – 14 000 IU/week orally for 6 months. Cytosolic Ca^{2+} measurements were performed by Fluo - 3 fluorimetry in isolated PBMCs. To determine the $P2X_7R$ function, a highly selective antagonist (AZ11645373) and the most potent agonist (BzATP) were used. The function of $P2X_7$ pores was measured by ethidium uptake at basal conditions and after stimulation or inhibition. The expression of surface $P2X_7R$ was evaluated by flow cytometry using the antibody to this receptor (anti-$P2X_7$ extracellular).

STATISTICAL ANALYSES

All values are expressed as means ± SD. Statistical analysis was carried out by the SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to evaluate a sample normality distribution. The statistical significance of differences was tested by the independent 2-population Student's *t*-test for normally distributed data and the Wilcoxon's test for a non-parametric analysis. A p-value < 0.05 was considered significant.

SUMMARY

Vitamin D_3 supplementation

- reduced $[Ca^{2+}]_i$ in PBMCs of early CKD
- had inhibitory effect on calcium entry through $P2X_7$ channels
- had no effect on permeability of $P2X_7$ pores
- reduced expression of surface $P2X_7$ receptors by 55%

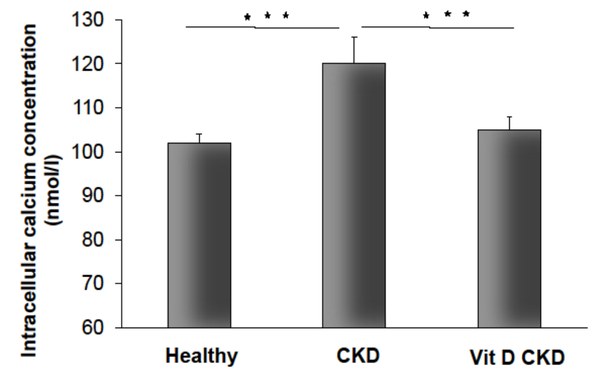
RESULTS

Free cytosolic calcium ($[Ca^{2+}]_i$) and $25(OH)D_3$ concentrations before and after cholecalciferol supplementation in CKD patients

Parameters	Baseline	6 month
sGFR (ml/s)	1.08 ± 0.09	1.05 ± 0.07
$25(OH)D_3$ (ng/ml)	18 ± 2	35 ± 2 ***
$[Ca^{2+}]_i$ (nmol/l)	120 ± 2	105 ± 1 ***

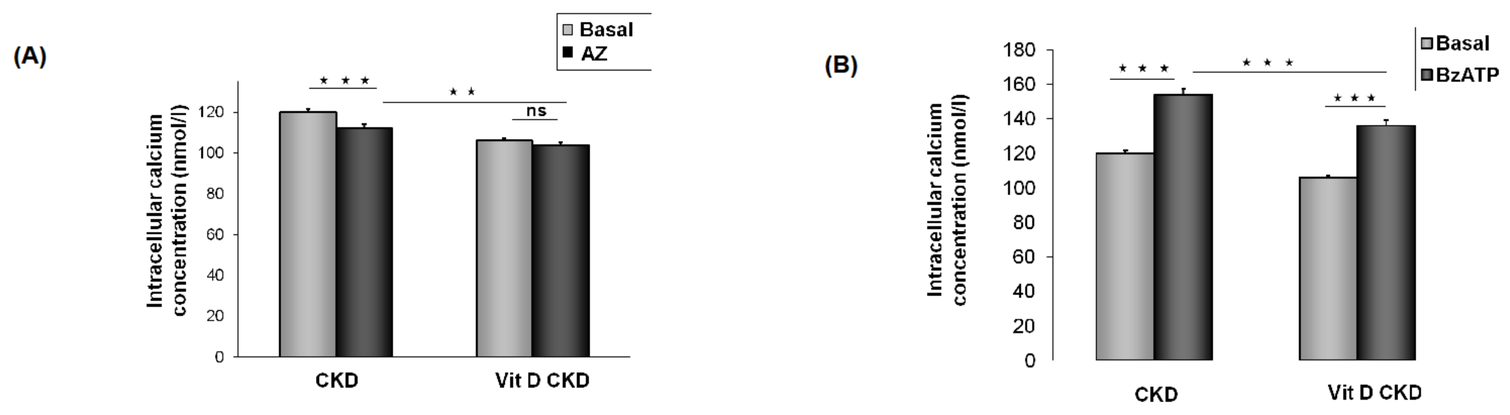
Values are expressed as mean ± SEM. *** P<0.001 for comparison with baseline.

Initial $25(OH)D_3$ concentrations were low and significantly increased after the vitamin D_3 supplementation reaching the recommended level above 30 ng/ml.The $[Ca^{2+}]_i$ in PBMCs significantly decreased after the 6 - month vitamin D_3 supplementation to values comparable with those in healthy subjects.



Cytosolic Ca^{2+} measurements of healthy volunteers and CKD patients before and after vitamin D_3 supplementation (***P< 0.001).

Function of $P2X_7$ channels

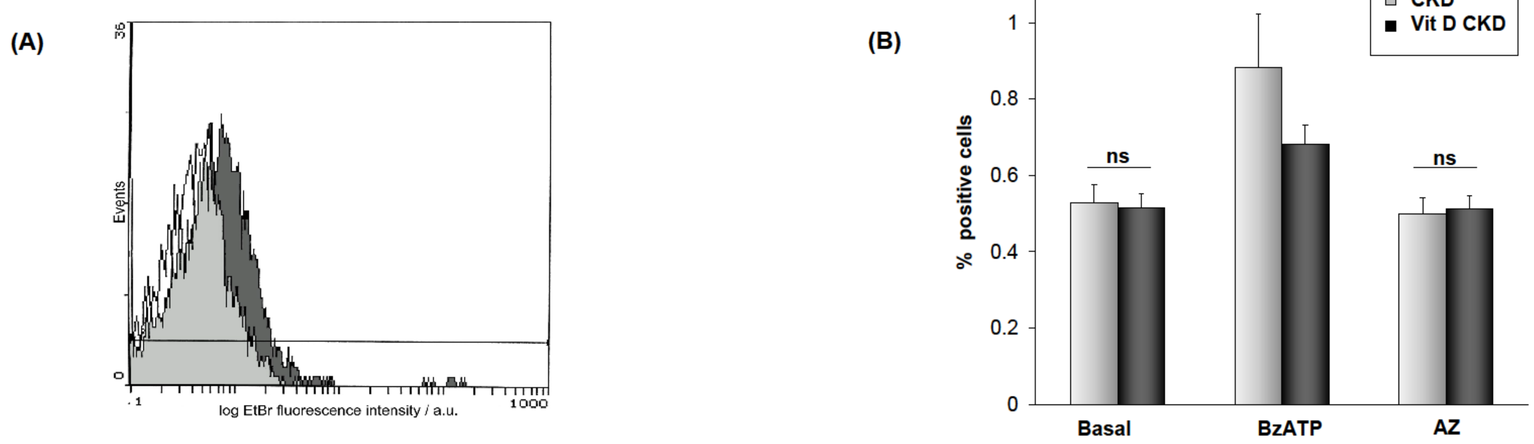


(A) The application of $P2X_7R$ antagonist AZ11645373 on PBMCs of CKD patients led to reduction in $[Ca^{2+}]_i$, but no effect was found after the vitamin D_3 supplementation (***P < 0.001, **P < 0.01).

(B) The agonist of purinergic $P2X_7R$ (BzATP) caused a sustained increase in $[Ca^{2+}]_i$ in PBMCs of CKD patients and also after the vitamin D_3 supplementation, however did not reach the values of $[Ca^{2+}]_i$ before supplementation (***P < 0.001).

These results demonstrate the inhibitory effect of vitamin D_3 on calcium entry through $P2X_7$ channels.

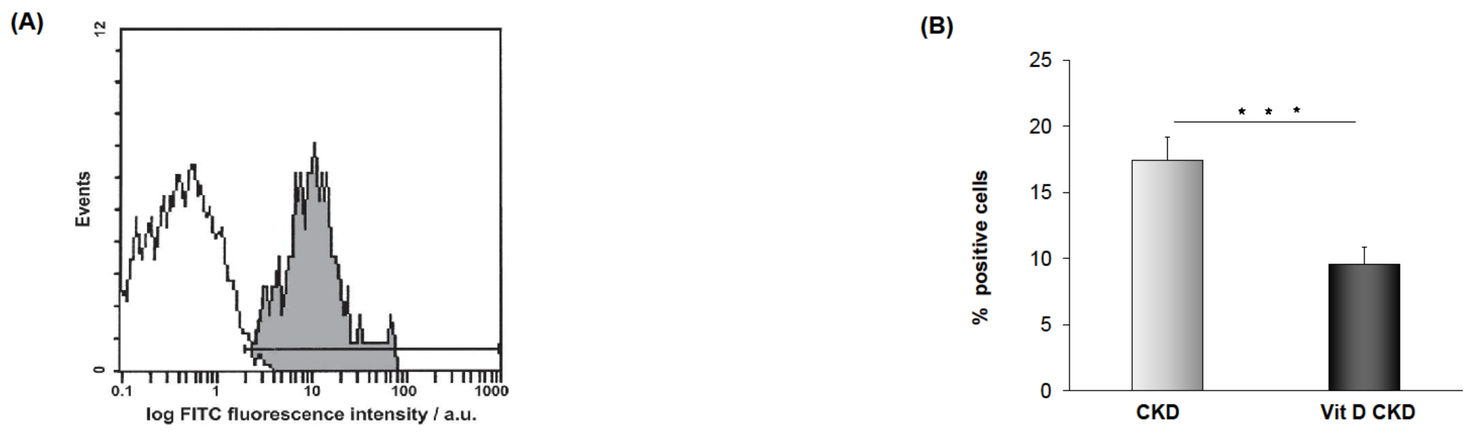
Permeability of $P2X_7$ pores



(A) Representative flow cytometry histograms of ethidium bromide entry into PBMCs of a CKD patient at basal conditions (white peak) and after stimulation by BzATP (50 μmol/l, (gray peak).

(B) The vitamin D_3 supplementation did not change the permeability of $P2X_7$ pores after the application of either agonist (BzATP) or antagonist (AZ11645373) of $P2X_7$Rs.

Expression of cell surface $P2X_7$Rs



(A) Representative flow cytometry histograms of PBMCs immunostained with primary antibody for the extracellular domain of the $P2X_7R$ (gray peak) and an isotype-matched control (Ig2a, white peak).

(B) The expression of surface $P2X_7$Rs decreased in the whole population of PBMCs by 55% after vitamin D_3 supplementation (***P < 0.001).

CONCLUSION

These results demonstrate the inhibitory effect of vitamin D_3 supplementation on pro-inflammatory $P2X_7R$ channels and $P2X_7$Rs expression already in early stages of CKD. This might be one of the mechanisms of an immunomodulatory effect of vitamin D.

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