

Immunomodulatory properties of cholecalciferol in rats with experimentally induced inflammation

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Abstract

The study aimed to investigate anti-inflammatory and immunomodulatory effects of cholecalciferol (vitamin D₃) in rats with complete Freund's adjuvant-induced arthritis (AIA) and lipopolysaccharide (LPS)-induced inflammation. In the first experiment, rats were treated with cholecalciferol 14 days before or from the day of induction of arthritis. In the second set-up, animals received cholecalciferol for 14 days which was followed by LPS injection. TNF (tumour necrosis factor)-alpha, IL (interleukin)-1β, TGF (transforming growth factor)-β1 levels were measured by enzyme linked immunosorbent assay (ELISA). Cholecalciferol treatment reduced paw oedema and ankle joint diameter in AIA. Significantly lower IL-1β concentrations were found in cholecalciferol-treated arthritic rats. In LPS-challenged rats, cholecalciferol markedly lowered serum TNF-α, whereas an elevation in IL-1β concentrations was observed. Cholecalciferol slightly increased TGF-β1 serum concentration in arthritic rats and non-significantly reduced its level in LPS-challenged animals. Our findings showed that cholecalciferol exerts immunomodulatory properties which probably contribute to its anti-inflammatory effect.

Keywords

arthritis, cholecalciferol, cytokines, inflammation, lipopolysaccharide

Introduction

Vitamin D is recognised as a crucial dietary component with diverse physiological functions (Bilezikian et al. 2021). It functions more akin to a prohormone than a conventional vitamin. The hormonally active form, calcitriol, acts through the vitamin D nuclear receptor (VDR) to exert its effects (Mayne and Burne 2019). Beyond its role in

calcium and phosphate homeostasis, vitamin D regulates immune response, cellular proliferation and differentiation, lipid metabolism etc. (Christakos et al. 2016; Nikolova et al. 2019).

Vitamin D deficiency is a major health problem worldwide. It is associated not only with disorders related to disrupted calcium homeostasis, such as rickets, osteomalacia and increased risk of dental caries (Minisola et al. 2020;

Stoichkov et al. 2023). Emerging evidence implicates vitamin D deficiency in conditions such as metabolic syndrome, obesity, sarcopenia, chronic inflammation, autoimmune disorders, cancer etc. (Holick 2017; Nikolova et al. 2018b). Extensive research underscores the pleiotropic functions of vitamin D, including antioxidative, anti-inflammatory, immunomodulatory and anticancer activities (Christakos et al. 2016).

Vitamin D plays an essential role in the regulation of immune and inflammatory processes. Its deficiency might be associated with chronic inflammation, evidenced by negative correlation between low 25-hydroxyvitamin D levels and serum C-reactive protein (CRP) (Nikolova et al. 2018a). Vitamin D modulates cell proliferation and inhibits the release of inflammatory cytokines, such as tumour necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-8 (IL-8) (Calton et al. 2015; Zheng et al. 2020).

VDR is present in immune cells and vitamin D plays a crucial role in regulating both innate and adaptive immune responses. This vitamin acts by hindering the transformation of monocytes and macrophages into dendritic cells and by halting the generation of inflammatory cytokines. Moreover, vitamin D inhibits the multiplication of Th1, Th17 and B cells (Bizzaro et al. 2017). Vitamin D deficiency is associated with increased nuclear factor κ B (NF κ B) activity, a critical regulator of inflammation implicated in various chronic inflammatory conditions (Mousa et al. 2016). Experimental studies demonstrate that vitamin D supplementation mitigates arthritis and other inflammatory diseases (Moghaddami et al. 2012).

Inflammatory mediators, such as TNF- α and IL-1 β , are central to joint inflammation and bone deformities in rheumatoid arthritis (RA), showing an inverse correlation with vitamin D levels (Aslam et al. 2019). The intraplantar injection of complete Freund's adjuvant (CFA) induces an experimental arthritis model that closely resembles RA in humans, exhibiting similar clinical, histological and immunological characteristics (Hu et al. 2019). Lipopolysaccharide (LPS), derived from gram-negative microorganisms, is a potent pro-inflammatory molecule triggering significant inflammatory reactions (Xu et al. 2017). *In vivo*, a single LPS injection is a commonly employed method to induce inflammation, leading to the secretion of inflammatory cytokines, such as TNF- α , IL-1 β and IL-6 (Bossù et al. 2012).

The anti-inflammatory properties of vitamin D could be explained with its effect on expression of enzymes responsible for synthesis of pro-inflammatory mediators, influence on intracellular signalling cascades involved in inflammation and interaction with transcription factors essential for regulation of genes for production of pro-inflammatory and anti-inflammatory molecules (Wöbke et al. 2014). Since immune cells contain the enzymes required for synthesis of the active form of vitamin D, we aimed to investigate the immunomodulatory effect of cholecalciferol. Serum levels of widely distributed in inflammatory disorders pro-inflammatory cytokines (TNF- α , IL-1 β) and immunoregulatory

transforming growth factor beta 1 (TGF-beta 1) were studied in two inflammatory models – CFA induced arthritis and LPS model of acute inflammation.

Material and methods

Drugs and reagents

Cholecalciferol (Merck), lipopolysaccharide E. Coli O55 (Sigma Aldrich), complete Freund's adjuvant (Sigma Aldrich); rat IL-1 beta, TNF-alpha, TGF beta-1 ELISA kits (Diacalone); rat vitamin D₃ ELISA kit (MyBioSource).

Ethical statement

All experiments were conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes. Permission for this study was obtained from the Ethics Committee at the Medical University of Plovdiv, Bulgaria, under protocol № 1/13.02.2020, as well as from the Animal Health and Welfare Directorate of the Bulgarian Food Safety Agency, permit № 249/22.11.2019.

Animals and experimental design

In our study, we used adult male Wistar rats weighing 200 ± 20 grams. They were housed in a controlled laboratory setting with a 12-hour light-dark cycle, maintained at a room temperature of 22 ± 2 °C and air humidity of $55 \pm 5\%$. Tap water and food were available *ad libitum*. Prior to the experiments, the rats were acclimatised to the laboratory conditions and all experiments were conducted during the daytime.

To assess the anti-inflammatory effects of cholecalciferol in rats with CFA-induced arthritis, the animals were randomly divided into six groups (n = 8):

Group 1: control group: olive oil 0.1 ml/kg bw,

Group 2: positive control group: olive oil 0.1 ml/kg bw + CFA,

Group 3: cholecalciferol 500 IU/kg bw (pretreatment) + CFA,

Group 4: cholecalciferol 1000 IU/kg bw (pretreatment) + CFA,

Group 5: cholecalciferol 500 IU/kg bw + CFA,

Group 6: cholecalciferol 1000 IU/kg bw + CFA.

Groups 1 to 4 were pretreated with the respective substance via oral gavage for 14 days. Groups 5 and 6 received their first dose of cholecalciferol on the day of CFA injection. Experimental arthritis was induced via a single intraplantar injection of 0.1 ml CFA into the right hind paw (day 0). The cholecalciferol treatment continued for 30 days, post arthritis induction, for all animals. At the end of the experiment (day 30), blood samples were taken for serum cytokine measurement.

To evaluate the anti-inflammatory effect of cholecalciferol in rats with LPS-induced systemic inflammation, the animals were randomly divided into four groups (n = 8):

Group 1: control group: olive oil 0.1 ml/kg bw,
 Group 2: positive control group: olive oil 0.1 ml/kg bw + LPS,
 Group 3: cholecalciferol 500 IU/kg bw + LPS,
 Group 4: cholecalciferol 1000 IU/kg bw + LPS.

All animals were pretreated orally, for 2 weeks, with respective substances. On day 15, LPS was intraperitoneally administered at a dose of 1 mg/kg bw to groups 2, 3 and 4. Blood samples for immunological assays were collected four hours, post-administration. In both experiments, the control groups were treated with olive oil, as it served as the diluent for cholecalciferol.

Anti-inflammatory tests

Digital Water Plethysmometer (Ugo Basile, Italy) was employed to assess the CFA-induced inflammatory response, as outlined by Cong et al. (2015). Hind paw swelling volume was measured before the CFA injection (day 0) and on days 1, 15 and 30 post arthritis induction. The displacement of water resulting from immersion of the hind paw into the measuring tube was transferred to a second tube, causing volume displacement and this value was recorded. The percentage of anti-inflammatory action was then calculated using the following equation:

$$\% \text{ Inhibition of paw volume} = \frac{PV_t - PV_0}{PV_0} \times 100$$

where PV_0 is the initial paw volume, PV_t is the paw volume at days 1, 15 and 30 following the CFA injection.

Ankle joint diameters were assessed using a digital caliper both before the CFA injection (day 0) and subsequently on days 1, 4, 6, 8, 11, 14, 18, 21, 25, 27 and 30.

Immunological assay

Serum levels of IL-1 β , TNF- α and TGF beta-1 were measured on days 30 and 15 of the respective experiment using solid-phase ELISA. The assessments were conducted in accordance with the manufacturer's instructions. Absorbance readings were taken at 450 nm using an ELISA reader and the absorbance values were then converted to concentrations (pg/ml) using a standard curve. The detection limits for IL-1 β , TNF- α and TGF beta-1 were 4.4 pg/ml, 15 pg/ml and 48 pg/ml, respectively. Additionally, the detection limit for vitamin D₃ was 1.13 pg/ml.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19.0. All data are presented as mean \pm SEM (standard error of the mean). The data were analysed using repeated measures one-way ANOVA, followed by the

Tukey post hoc test. A value of $p < 0.05$ was accepted for statistically significant.

Results

1. Effects of cholecalciferol on inflammation in CFA-induced arthritis

1.1. Plethysmometer: all experimental groups, as well as the positive control, notably increased paw volume compared to the control rats on day 1 ($p < 0.001$), day 15 ($p < 0.001$ for groups 2 and 5; $p < 0.05$ for groups 3, 4 and 6) and day 30 ($p < 0.001$ for groups 2, 5 and 6; $p < 0.05$ for groups 3 and 4). Animals pretreated with cholecalciferol in both doses significantly inhibited paw oedema on the 15th and 30th days compared to the positive control ($p < 0.05$ and $p < 0.001$, respectively) (Fig. 1).

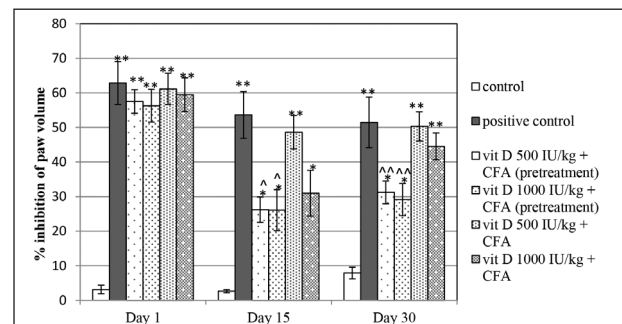


Figure 1. Effect of cholecalciferol on paw volume in rats with CFA-induced arthritis. Data are expressed as mean \pm SEM. * $p < 0.05$ compared with control; ** $p < 0.001$ compared with control; ^ $p < 0.05$ compared with positive control; ^^ $p < 0.001$ compared with positive control.

1.2. Ankle joint diameters: throughout all testing days, all experimental groups and the positive control exhibited significantly increased joint diameters compared to the control ($p < 0.01$). Animals pretreated with cholecalciferol at a dose of 500 IU/kg bw notably decreased joint diameter on days 14 and 18 compared to the positive control ($p < 0.01$ and $p < 0.05$, respectively). Rats pretreated with the higher dose of vitamin D₃ significantly reduced ankle diameter on days 11 ($p < 0.01$), 14 ($p < 0.01$), 18 ($p < 0.001$), 21 ($p < 0.05$), 25 ($p < 0.01$), 27 ($p < 0.05$), and 30 ($p < 0.001$). Animals that began cholecalciferol (1000 IU/kg bw) treatment on the day of CFA injection showed a significant decrease in joint diameter on days 11 and 14 compared to the positive control ($p < 0.01$) (Table 1).

2. Effects of cholecalciferol on pro- and anti-inflammatory cytokine serum levels

2.1. TNF- α , IL-1 β , TGF- β 1 and vitamin D₃ serum levels in rats with CFA-induced arthritis TNF- α : the positive control and all animals, except those pretreated with 1000 IU/kg cholecalciferol, exhibited significantly higher levels of TNF- α compared to the control group ($p < 0.05$). Rats

Table 1. Effect of cholecalciferol on ankle joint diameter in rats with CFA-induced arthritis.

Day	control	positive control	vit D 500IU/kg + CFA (pretreatment)	vit D 1000 IU/kg + CFA (pretreatment)	vit D 500IU/kg + CFA	vit D 1000 IU/kg + CFA
1	5.18 ± 0.12	8.96 ± 0.16*	9.37 ± 0.11*	9 ± 0.14*	9 ± 0.15*	8.93 ± 0.16*
4	5.12 ± 0.12	9.62 ± 0.14*	9.81 ± 0.19*	9.81 ± 0.16*	10.37 ± 0.21*	8.87 ± 0.17*
6	5.12 ± 0.12	8.62 ± 0.17*	9.18 ± 0.17*	8.87 ± 0.18*	8.68 ± 0.16*	8.25 ± 0.16*
8	5.37 ± 0.15	8 ± 0.18*	8.62 ± 0.18*	8.18 ± 0.2*	8 ± 0.14*	7.81 ± 0.14*
11	5.51 ± 0.1	9.1 ± 0.12*	8.53 ± 0.19*	8.01 ± 0.2* ^^	8.51 ± 0.14*	7.63 ± 0.14* ^^
14	5.53 ± 0.11	8.71 ± 0.13*	7.88 ± 0.15* ^^	7.93 ± 0.18* ^^	8.53 ± 0.15*	7.87 ± 0.15* ^^
18	5.51 ± 0.09	8.11 ± 0.1*	7.52 ± 0.13* ^	6.87 ± 0.14* ^^	7.93 ± 0.1*	7.56 ± 0.11*
21	5.6 ± 0.16	7.78 ± 0.1*	7.56 ± 0.13*	6.87 ± 0.13* ^	8.06 ± 0.13*	7.68 ± 0.12*
25	5.6 ± 0.16	7.56 ± 0.14*	7.25 ± 0.17*	6.26 ± 0.16* ^^	8.12 ± 0.14*	7.75 ± 0.14*
27	5.62 ± 0.16	7.65 ± 0.12*	7.31 ± 0.18*	6.16 ± 0.14* ^^	8.21 ± 0.18*	7.81 ± 0.13*
30	5.43 ± 0.27	7.68 ± 0.1*	7.56 ± 0.19*	6.1 ± 0.12* ^^	7.87 ± 0.1*	7.62 ± 0.12*

Data are expressed as mean ± SEM. *p < 0.01 compared with control; ^p < 0.05 compared with positive control; ^^p < 0.01 compared with positive control; ^^p < 0.001 compared with positive control.

pretreated with vitamin D₃ notably decreased serum levels of TNF-α compared to the positive control (p < 0.05 and p < 0.01, respectively). Animals receiving a higher dose of vitamin D from the day of AIA induction also significantly decreased TNF-α concentration compared to the positive control (p < 0.05) (Fig. 2).

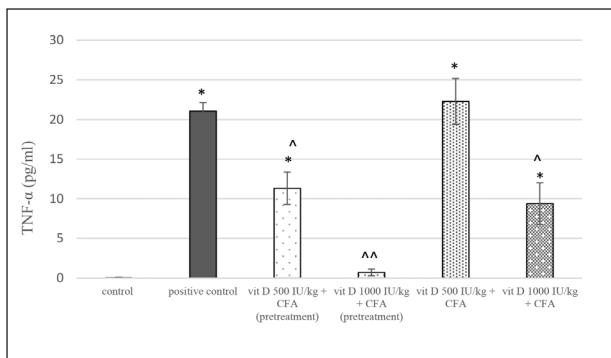


Figure 2. Effect of cholecalciferol on TNF-α serum levels in rats with CFA-induced arthritis. Data are expressed as mean ± SEM. *p < 0.05 compared with control; ^p < 0.05 compared with positive control; ^^p < 0.01 compared with positive control.

IL-1β: animals treated solely with CFA exhibited a notable increase in IL-1β serum levels compared to the control group (p < 0.01). All experimental groups showed significantly lower levels of IL-1β compared to the positive control (p < 0.05) (Fig. 3).

TGF-β1: neither control group exhibited a significant difference in serum levels of TGF-β1. Cholecalciferol treatment led to a slight increase in the concentration of this cytokine, but none of the experimental groups reached significance when compared with either of the control animals (Fig. 4).

Vitamin D₃: serum levels of cholecalciferol were insignificantly lower in rats with AIA compared to the control group. However, cholecalciferol supplementation resulted in an increase in its serum concentration. Significance was observed in rats pretreated with both doses of vitamin D3 compared to both control groups (p < 0.05 and p < 0.01, respectively). Rats receiving the higher dose of cholecalciferol from the day of arthritis induction exhibit-

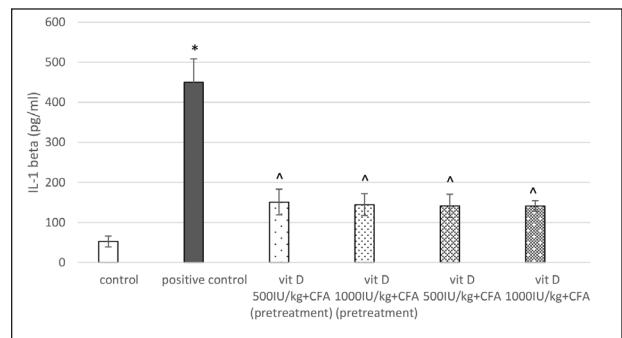


Figure 3. Effect of cholecalciferol on IL-1β serum levels in rats with CFA-induced arthritis. Data are expressed as mean ± SEM. *p < 0.01 compared with control; ^p < 0.05 compared with positive control.

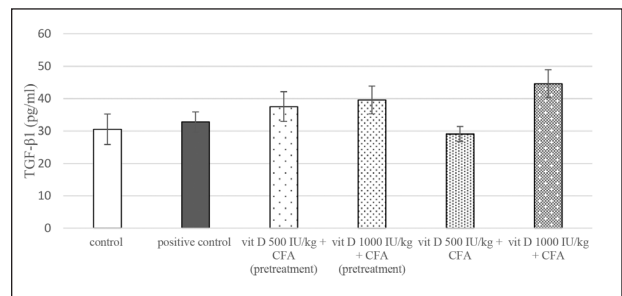


Figure 4. Effect of cholecalciferol on TGF-β1 serum levels in rats with CFA-induced arthritis. Data are expressed as mean ± SEM.

ed a significant increase in its level compared to the positive control (p < 0.05) (Fig. 5).

The obtained concentrations of cholecalciferol were higher compared with non-treated rats, but remained within the normal range (maximum 181.42 ± 6.8 ng/ml) for related states without reaching toxic levels (Wöbke et al. 2014).

2.2. TNF-α, IL-1β, TGF-β1 and vitamin D₃ serum levels in rats with LPS-induced systemic inflammation.

TNF-α: both the positive control (p < 0.01) and the two experimental groups (p < 0.01 and p < 0.05) significantly increased serum levels of TNF-α compared to the control group. Rats treated with the higher dose of cholecalciferol exhibited significantly lower serum levels of TNF-α compared to animals that received only LPS (p < 0.05) (Fig. 6).

IL-1 β : animals injected solely with LPS, as well as the two experimental groups receiving cholecalciferol, exhibited a notable elevation in serum levels of IL-1 β compared to the control rats ($p < 0.001$) (Fig. 7).

TGF- β 1: rats from the positive control group showed a significant increase in serum levels of TGF- β 1 compared to the control group ($p < 0.05$). However, animals from the two groups treated with vitamin D₃ at doses of 500 and 1000 IU/kg bw exhibited a decrease in serum concentration of TGF- β 1, although this change did not reach statistical significance (Fig. 8).

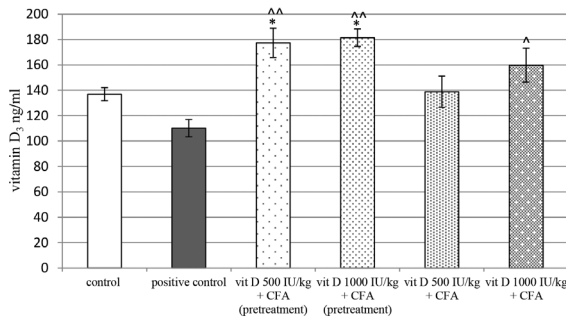


Figure 5. Serum levels of vitamin D₃ in rats with CFA-induced arthritis. Data are expressed as mean \pm SEM. * $p < 0.05$ compared with control; $\wedge p < 0.05$ compared with positive control; $\wedge\wedge p < 0.01$ compared with positive control.

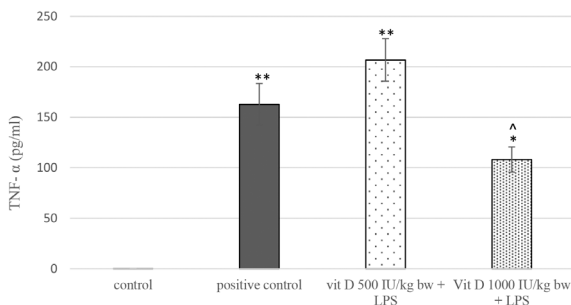


Figure 6. Effect of cholecalciferol on TNF- α serum levels in rats with LPS-induced systemic inflammation. Data are expressed as mean \pm SEM. * $p < 0.05$ compared with control; ** $p < 0.01$ compared with control; $\wedge p < 0.05$ compared with positive control.

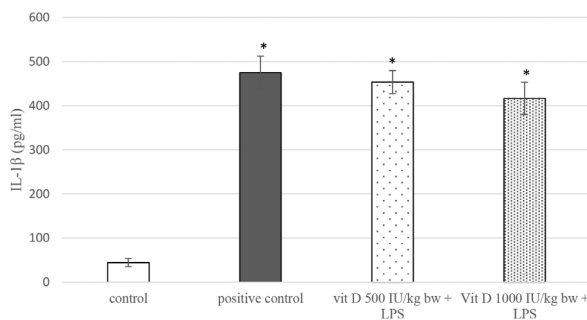


Figure 7. Effect of cholecalciferol on IL-1 β serum levels in rats with LPS-induced systemic inflammation. Data are expressed as mean \pm SEM. * $p < 0.001$ compared with control.

Vitamin D₃: administration of LPS led to a slight reduction in cholecalciferol serum levels compared with the control group. However, both experimental groups exhibited a significant increase in vitamin D serum levels compared with the positive control ($p < 0.05$ and $p < 0.01$, respectively) (Fig. 9).

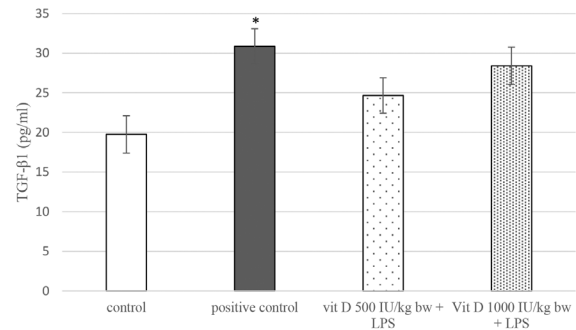


Figure 8. Effect of cholecalciferol on TGF- β 1 serum levels in rats with LPS-induced systemic inflammation. Data are expressed as mean \pm SEM. * $p < 0.05$ compared with control.

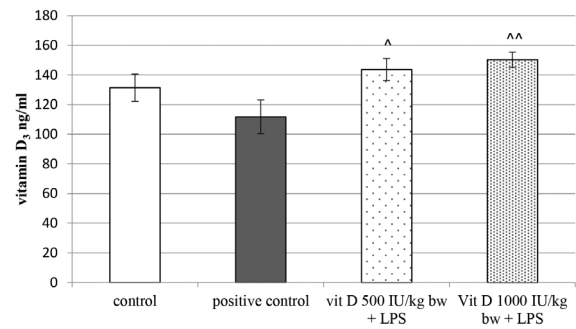


Figure 9. Serum levels of vitamin D₃ in rats with LPS-induced systemic inflammation. Data are expressed as mean \pm SEM. $\wedge p < 0.05$ compared with positive control; $\wedge\wedge p < 0.01$ compared with positive control.

Discussion

Beyond its role in maintaining calcium-phosphorus homeostasis, vitamin D plays a pivotal role in both innate and adaptive immunity. Deficiency in this vitamin has been linked to an increased risk of autoimmune and inflammatory disorders. The principal finding of our current study is that cholecalciferol suppresses inflammation and demonstrates immunomodulatory effects in two distinct experimental models: adjuvant-induced arthritis (an autoimmune-mediated chronic inflammatory process) and LPS-induced acute systemic inflammation.

Vitamin D deficiency has been observed in patients with various autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type 1 diabetes mellitus, multiple sclerosis and thyroiditis (Bizzaro et al. 2017). Low serum levels of vitamin D correlate positively with the onset and severity of

rheumatoid arthritis (Aslam et al. 2019). Our study's results demonstrate that cholecalciferol significantly inhibits the inflammatory response and reduces the diameter of affected ankle joint. Administering vitamin D₃ before arthritis induction exhibited a more pronounced beneficial effect. These findings align with previous studies regarding the effect of vitamin D in experimentally induced arthritis. Vitamin D deficiency results in more severe T-cell-mediated experimental arthritis and an increased number of CD45+ cells in synovial tissues (Moghaddami et al. 2012). Sun et al. (2019) found that, in rats with adjuvant-induced arthritis, 1,25(OH)₂D₃ reduces arthritic score, synovial hyperplasia, inflammatory cell infiltration, mRNA expression of inflammatory cytokines and induces apoptosis of synoviocytes.

Treatment with vitamin D following CFA induction of arthritis has a protective effect, with a significant decrease in inflammatory markers (e.g. TNF- α , IL-6, rheumatoid factor), beneficial effects on serum lipids and an increase in red blood cells, haemoglobin, haematocrit and platelets (Gaafar et al. 2018). Our study's results indicate that preventative use of vitamin D is more effective than its application after arthritis initiation. The highest dose produced the best outcome regarding the inflammatory response.

Vitamin D could ameliorate inflammation in AIA through several mechanisms. A recent review discussed the role of small molecules, such as prostaglandins, leukotrienes, nitric oxide, reactive oxygen species and lipoxins in the pathogenesis of inflammation observed in rheumatoid arthritis (Cheng et al. 2021). Vitamin D regulates the expression of genes for cyclooxygenase and lipoxygenase and inhibits oxidative stress, effects that may contribute to its therapeutic effect in arthritis (Wöbke et al. 2014; Chen et al. 2019). Inflammatory cytokines, such as TNF- α , IL-1, IL-6 etc., play a central role in the development of joint inflammation and systemic features of rheumatoid arthritis (Christodoulou and Choy 2006). Hence, we further studied the influence of cholecalciferol administration on serum levels of TNF- α , IL-1 and TGF- β 1.

TNF- α plays a crucial role in inflammation's pathogenesis. It causes vasodilation by stimulating inducible nitric oxide (NO) synthetase, increases vascular permeability and expression of cell adhesion molecules and stimulates production of reactive oxygen species. TNF- α is primarily released by macrophages, but other cells can also produce this pro-inflammatory cytokine, such as neutrophils, NK-cells, B-lymphocytes etc. (Zelová and Hošek 2013). TNF- α is the most important pro-inflammatory cytokine in rheumatoid arthritis' pathogenesis (Noack and Miossec 2017). It is involved in articular erosion, bone destruction, pannus formation and nociceptor sensitisation increase. TNF- α stimulates synovial fibroblasts, endothelial cells, osteoclasts and promotes production of other pro-inflammatory cytokines (Brennan and McInnes 2008; Brzustewicz and Bryl 2015). AIA is characterised by CD4+ T-cells activation and increased production of TNF- α , other pro-inflammatory molecules and chemotactic agents (Billiau and Matthys 2001). It is used as an experimental model of autoimmune inflammation resembling rheumatoid arthritis. In our study, cholecalciferol significantly reduced TNF- α serum levels in rats with AIA.

TNF- α represents an important therapeutic target in treating RA. Apart from its use in RA patients, anti-TNF- α therapies are also beneficial in various other autoimmune disorders, including inflammatory bowel disease, ankylosing spondylitis and psoriasis (Lopetuso et al. 2018). However, currently available anti-TNF- α strategies are associated with risks of serious adverse clinical effects (Shivaji et al. 2019) and a significant portion of patients do not demonstrate a good therapeutic response. Therefore, vitamin D supplementation may improve the efficacy of anti-TNF- α therapies by targeting this cytokine and reducing its production.

Bacterial LPS is one of the primary triggers of TNF- α synthesis. LPS is a component of the outer membrane of Gram-negative bacteria. It stimulates the innate immune response and causes a robust inflammatory reaction (Wali et al. 2020). LPS binds to Toll-like receptor 4 (TLR4), leading to activation of mitogen-activated protein kinase (MAPK) and NF- κ B. This results in increased production of pro-inflammatory cytokines, including TNF- α (Mazgaen and Gurung 2020). Cholecalciferol significantly reduced TNF- α serum levels in LPS-challenged rats. Several *in vitro* studies have shown that vitamin D suppresses LPS-induced TNF- α production (Panichi et al. 1998; Giovannini et al. 2001; Chen et al. 2019). Our study's results confirm this effect in *in vivo* conditions. The reduced cholecalciferol serum levels observed in LPS-challenged control rats are likely due to its conversion to the active form, which participates in regulating TNF- α synthesis. The probable mechanisms involved in the inhibitory effect of vitamin D on TNF- α are inhibition of MAPK and NF- κ B. It has been demonstrated that both the active form of vitamin D and 25-hydroxyvitamin D₃ inhibit p38 MAPK by up-regulating mitogen-activated protein kinase phosphatase-1 (Zhang et al. 2012). A recent study showed that 1,25(OH)₂D₃ inhibits the activation of the NF- κ B signal pathway (Tian et al. 2018). Sadeghi et al. demonstrated that vitamin D down-regulates the expression of toll-like receptor 2 (TLR2) and TLR4 in human monocytes with subsequent decrease in TNF- α following LPS challenge (Sadeghi et al. 2006).

IL-1 β is an important molecule implicated in the development of autoinflammatory and autoimmune diseases. It has also been shown to play a role in the pathogenesis of ischemic injury in stroke, type 2 diabetes due to its cytotoxic effects on pancreatic beta cells, osteoarthritis, gout, myeloma and heart failure following myocardial infarction. Blockade of IL-1 β signal pathways results in an improvement of the condition in the above-mentioned diseases (Dinarello 2011). In RA, IL-1 β induces bone resorption and cartilage destruction by stimulating osteoclastogenesis and release of matrix metalloproteinases (Noack and Miossec 2017). Experimental studies showed that mice, deficient in the IL-1 receptor antagonist, spontaneously develop polyarthritis with increased levels of autoantibodies (Horai et al. 2000). A recent *in vivo* study demonstrated that vitamin D reduces the production of IL-1 β and other pro-inflammatory cytokines from whole blood-derived macrophages of patients with RA (Neve et al. 2014). Mateen et al. (2017) found that 25-hydroxy vitamin D levels are lower in patients with

RA and this was associated with increased concentrations of pro-inflammatory cytokines, including IL-1 β . Our study's results showed that cholecalciferol reduces serum IL-1 β in rats with AIA. This effect was observed independently of the dose and duration of treatment, in animals pretreated with vitamin D, as well as in those that received cholecalciferol from the day of induction of arthritis.

IL-1 β is mainly produced by monocytes and macrophages (Lopez-Castejon and Brough 2011). Several *in vitro* studies investigated the effect of vitamin D on IL-1 β production by these cells. However, existing data about the effect of vitamin D on IL-1 β expression are controversial. Lee et al. showed that 1,25(OH) $_2$ D $_3$ increases its RNA expression and production in human monocyte-derived macrophages and this effect was enhanced in the presence of LPS (Lee et al. 2011). Earlier studies also demonstrated that vitamin D increases IL-1 β production by monocytes and macrophages (Bhalla et al. 1986). By contrast, Villaggio et al. found that the active form of the vitamin down-regulates IL-1 β release in macrophages (Villaggio et al. 2012). The results from the current study support the idea that vitamin D may increase IL-1 β production in the settings of acute inflammation. A significant elevation in serum levels of this cytokine was observed in LPS-challenged rats. Di Rosa et al. found that, in freshly-isolated human monocytes treated with 1,25(OH) $_2$ D $_3$ and LPS, a marked increase in IL-1 β expression is observed, whereas, in macrophages cultured for seven days, vitamin D inhibits the expression of this cytokine (Di Rosa et al. 2012). This agrees with the results obtained in our *in vivo* study and could explain the opposing effect of cholecalciferol on IL-1 β levels in LPS-challenged rats and those with AIA.

TGF- β is produced by a variety of cells, including non-immune cells and has pleiotropic effects in the body. It is important for the development and maintenance of immune tolerance, but may also promote inflammation and enhance autoimmune reactions (Sanjabi et al. 2009). TGF- β signalling induces immune tolerance and suppresses T cell response by promoting development of thymic and peripheral tissue regulatory T cells (Liu et al. 2018). It was found that TGF- β may reduce bone and cartilage damage (Brzustewicz and Bryl 2015) in RA. This is one of the main cytokines during the remission of collagen-induced arthritis and probably has a role in limiting the duration of inflammatory response in this setting (Marinova-Mutafchieva et al. 2006). Our findings showed that vitamin D slightly increased serum TGF- β 1 levels in rats with AIA, which may contribute to its beneficial effect in reducing inflammation.

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LPS markedly increased TGF- β 1 serum levels. Our results agree with early *in vivo* studies about the stimulatory effect of LPS on the production of this cytokine from murine macrophages (Marriott and Bost 1998). Cholecalciferol insignificantly reduced serum concentrations of this cytokine in LPS-challenged animals. In systemic inflammation associated with massive cytokine release, TGF- β probably favours Th17 cell differentiation and has a pro-inflammatory role. Zhou L et al. (2008) showed that, in the presence of other cytokines with inflammatory action, TGF- β promotes Th17 lineage.

Conclusion

Cholecalciferol demonstrates significant immunomodulatory properties, which likely contribute to its anti-inflammatory effects. In both arthritic and LPS-challenged rats, vitamin D $_3$ significantly reduced TNF- α serum levels. However, the impact on IL-1 β and TGF- β 1 concentrations varied depending on the inflammatory model. Therefore, vitamin D supplementation may serve as a beneficial adjunct in the treatment of disorders associated with heightened TNF- α signalling. Cholecalciferol could exhibit a preventative effect in such diseases, as evidenced by the superior outcomes observed in animals pretreated with this vitamin.

Conflict of interest

The authors of this manuscript have declared that no conflict of interests exists.

Author contributions

Conceptualisation, ND, AM and IK; methodology, ND, AM, IK and MI; investigation, ND, AM, IK and MI; analysis, HZ and DD; writing, original draft preparation, AM, IK and HZ; writing, review and editing, IK and HZ; supervision, IK and DD.

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