Original Article

Vitamin D₃ Exerts Immunomodulatory and Memory Improving Properties in Rats with Lipopolysaccharide-Induced Inflammation

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Abstract

Introduction: Vitamin D is a fat-soluble secosteroid, its primary function being regulation of calcium-phosphate homeostasis and maintenance of bone integrity and mineralization. Recently, pleotropic effects of this vitamin have been recognized, including an immunomodulatory role and involvement in normal brain development and functioning.

Aim: The aim of the present study was to investigate the influence of cholecalciferol on serum inflammatory markers and memory functions in lipopolysaccharide (LPS) model of inflammation.

Materials and methods: Male Wistar rats were randomly divided into 4 groups (n=8): control group, LPS control group, LPS + cholecalciferol (vitamin D₃) 500 UI group, and 1000 IU/kg bw group. Step-down passive avoidance test, novel object recognition test (NORT), Y- and T-maze were performed to assess the memory functions. Latency, recognition index (RI), % spontaneous alteration (SA), and working memory index were registered. Tumor necrosis factor-alpha (TNF-α), IL-1β, transforming growth factor-β1 (TGF-β1), and brain derived neurotrophic factor (BDNF) serum levels were measured by ELISA.

Results: LPS administration caused significant impairment in memory functions in all memory tasks. Cholecalciferol treatment caused significant increase in % SA, RI, and working memory index. In the step-down passive avoidance test, cholecalciferol-treated groups showed statistically significant increase in latency in the long-term memory test. Vitamin D₃-treated rats showed decreased TNF-α and IL-1β serum levels whereas the concentration of TGF-β1 and BDNF increased.

Conclusions: Cholecalciferol improves spatial working and episodic memory, which can at least partially be explained with its effect on systemic inflammatory response that is closely related with the development of neuroinflammation.

Keywords

INTRODUCTION

Vitamin D is involved in a variety of physiological processes in our body. It exists in several forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol), which are precursors of the active form of vitamin D (calcitriol; 1,25-dihydroxycholecalciferol $(1,25(OH)_2D_3)$). Calcitriol is the hormonally active form of vitamin D_3, which is responsible for many of the effects of vitamin D by binding to nuclear vitamin D receptor protein (VDR).[1] These receptors have been located in almost all cell types, which may explain the vitamin D's various effects on different tissues. Its primary role is participation in calcium-phosphate homeostasis and maintaining bone integrity and mineralization. The pleiotropic effects of vitamin D include antimicrobial shielding, cardiovascular benefits, oxidative stress reduction, immunomodulation, neuroprotection, and anti-inflammatory action.[2] Vitamin D modulates cell proliferation and differentiation and plays an essential role in the responses of the nervous and immune systems.[3]

In the past few decades, several studies have demonstrated the correlation between vitamin D and brain health and the impact of its deficit on the brain and mental well-being. Recently, calcitriol has been recognized as a neuro-steroid with significant involvement in normal brain development and functioning.[4] The VDR's widespread distribution in brain tissues, with a great amount in motor, sensory, and limbic regions suggests that vitamin D takes part in the biosynthesis of neurotransmitters and neurotrophic factors.[5] Biosynthetic and metabolizing enzymes for calcitriol have also been detected in glial and neuronal cells.[6] Vitamin D promotes the synthesis of neurothrophic substances, such as brain-derived neurotrophic factor (BDNF) and prevents neuronal damage caused by oxidative stress and neuroinflammation.[7,8] Growing preclinical studies demonstrate that vitamin D has beneficial effects in different models of memory impairment. It has been shown that this neurosteroid improves memory in aging rats[9], animal models of Alzheimer's disease[10], and scopolamine-challenged rats[11]. Vitamin D deficiency is related to memory decline[12], cognitive impairment[13], neurological diseases[10], different types of dementia and autoimmune disorders[4]. The importance of vitamin D_3 in lowering the risk of these conditions climbs up due to the number of people having a significant vitamin D deficiency.

Vitamin D exerts key immunomodulatory action, with significant effect on innate and adaptive immunity. Different types of immune cells, (macrophages, T and B-lymphocytes, etc.) express VDR and are able to synthesize the active form of the vitamin $1,25(OH)_2D_3$ which modulates innate immune system via stimulation of the phagocytic activity of immune cells. Investigations on adaptive immune system show that vitamin D suppresses the production of pro-inflammatory cytokines from Th 1 cells and stimulates Th 2 cells.[14] In addition, $1,25(OH)_2D_3$ controls B lymphocytes' activity and the anti-inflammatory cytokines production by these cells.[15] Hypovitaminosis D stimulates systemic inflammation and enhances the expression of pro-inflammatory cytokines.[16]

TNF-α is a pleiotropic cytokine which is a key mediator of innate immunity and inflammatory response. In the CNS, it is involved in a variety of physiological functions like synaptic plasticity, ionic homeostasis, cognition, sleep, food and water intake, etc. Overproduction of TNF-α is implicated in the development of neuroinflammation, which is associated with neurodegenerative disorders like Alzheimer's disease.[17,18] Preclinical and clinical studies show that inhibition of TNF-α might improve memory and cognitive outcomes.[18] Interleukin-1β (IL-1β) and its receptor have been detected in the hippocampus.[19] IL-1 type 1 receptor is responsible for transduction of signals induced by IL-1β. This receptor is found under normal conditions in the hippocampal pyramidal neurons while its expression in glial cells is induced in the presence of injury.[20] Constitutive expression of IL-1 receptors in the hippocampus suggests that this cytokine plays an important role not only in neuroinflammation but is also involved in maintaining normal hippocampal function. Low levels of IL-1β can improve hippocampal-dependent learning and memory whereas high levels contribute to memory impairment.[21] Transforming growth factor β1 (TGF-β1) is constitutively found in brain structures involved in memory and learning like the cortex and the hippocampus.[22] Its expression undergoes up-regulation in case of brain injury as TGF-β1 has a neuroprotective role in the CNS. It protects neurons against apoptotic and glutamate NMDA receptor-mediated excitotoxic neuronal death.[23] TGF-β1 regulates the degree of microglial activation and prevents development of neurodegeneration.[24] It has been demonstrated that this growth factor stimulates microglial degradation of β-amyloid and its deficiency might be involved in the pathogenesis of neurodegeneration in patients with Alzheimer disease. Thus, therapeutic strategies that increase TGF-β1 signaling may be beneficial in preventing this disorder.[22]

Lipopolysaccharide (LPS) is an endotoxin with bacterial origin, a major component of the outer membranes of the cell walls of gram-negative bacteria. The intraperitoneal injection of LPS results in systemic inflammation followed by neuroinflammation and neurodegeneration.[25] LPS stimulates microglia leading to increased pro-inflammatory cytokine (TNF-α, IL-1, IL-6, etc.) levels in the central nervous system, which may result in brain inflammation and memory decline.[26]

Existing data show that vitamin D is capable of decreasing brain TNF-α and IL-1β expression in rat models of neuroinflammation and neurodegeneration.[8,27] It also up-regulates the expression of TGF-β1 and therefore regulates the production of pro- and anti-inflammatory cytokines.[28] We suggested that cholecalciferol by affecting peripheral cytokine production may prevent the development of neuroinflammation and improve memory functions in an experimental model of LPS-induced inflammation. Thus, the memory improving effect of vitamin D might directly be related to its immunomodulatory properties. Chole-

calciferol is the preferred form for studying the effect of vitamin D on inflammation because immune cells contain the enzymes responsible for its hydroxylation to the active form.[14]

AIM

The aim of the present study was to investigate the influence of cholecalciferol on serum inflammatory markers, BDNF, and memory functions in LPS model of inflammation.

MATERIALS AND METHODS

Ethical statement

All experimental procedures were performed in agreement with the European Convention for protection of Vertebrate Animals used for Experimental and other scientific purposes. For the present study, permission was obtained from the Ethics Committee at Medical University of Plovdiv, protocol No. 1/13.02.2020 and Animal Health and Welfare Directorate of the Bulgarian Food Safety Agency, permit No. 249/22.11.2019.

Drugs and reagents

Cholecalciferol (Merck), lipopolysaccharide E. Coli O55 (Sigma Aldrich). Rat BDNF ELISA Kit (Abbexa); Rat IL-10, TNF-α and TGF-β1 ELISA kits (Diaclone) were used for measurement of serum cytokine levels.

Animals

Adult male Wistar rats (200±20 g body weight) were used. They were housed in standard cages under controlled laboratory conditions (08:00-20:00 light-dark cycle, temperature 22±1ºC and humidity 55±5%). Food and water access were ad libitum.

Experimental design

Animals were randomly divided into 4 groups (n=8) as follows: a control group: the animals were treated with olive oil 0.1 ml/100 g bw as olive oil was used to dilute the cholecalciferol solution; LPS control group: animals were treated with LPS 250 mcg/kg bw intraperitoneally, the cholecalciferol 500 IU bw + LPS 250 mcg/kg bw group, and the cholecalciferol 1000 IU + LPS 250 mcg/kg bw group.

Rats were treated with cholecalciferol via oral gavage two weeks before and throughout the experiment. LPS was injected intraperitoneally in 5 consecutive days (from day 8 to day 12) and 30 min before the memory tasks. At the end of the experiment, blood samples were collected for immunological assay.

Behavioral tests

Step-through inhibitory "passive" avoidance test

Two-compartment apparatus, consisting of one dark and one light chamber, brightly illuminated, connected by a sliding automatic door, was used (UgoBasile, Italy). The experiment consists of a training session for the first two consecutive days, a short-memory trial at day 3, and a long-term memory test at day 10. During the learning sessions, the animal was placed in the light compartment facing away from the dark chamber to which it got access following a door delay of 7 second. After entering the dark compartment, the door slided down and the animal was subjected to a short-lasting aversive stimulus (electrical foot shock for 9 sec, intensity 0.4 mA). The time spent in the illuminated chamber was recorded as step-through latency in seconds. Both learning and memory retention sessions included 3 trials. During the memory retention tests, we used the same experimental set up but no shock was delivered to the animal. When the rat did not enter the dark compartment within 178 seconds, the trial was terminated.[29]

Elevated T-maze

T-maze was used to evaluate spatial working memory in rats. It has an upper-case T-shape design with a stem length of 50 cm, arm length of 40 cm and is situated 50 cm above ground. The task relies on either spontaneous or rewarded alternation. We used the latter one. The rats were left food deprived for 24 hours prior to the experiment. Each learning session involved 11 trials – an initial forced trial followed by 10 choice trials. During the forced trial, one arm was blocked and reward pellets were placed in the opposite arm, hence the animal was forced to enter the baited arm. During the choice trails, both arms were accessible, but the reward was available at the same place as in the initial trial. Throughout the choice trials, the animals had to stay away from the unexplored arm, which is their natural instinct, and enter the already familiar arm with reward pellets. The rat was positioned at the base of the T-shape and arm entries were recorded, when the whole rat (including tail) was in the arm. Inter-trial interval was set on 5 min. A working memory index was evaluated – number of correct choices out of the total number of trials.[30]

Y-maze

The Y-maze task and spontaneous alternations are widely used to assess spatial memory in rodents and is dependent on their natural exploratory instincts. This test is helpful in evaluating working memory, loco-motor activity, and stereotypical behavior. We used Y-maze set up constructed of black acrylic glass with three arms interconnected at 120°. The arms were equal (50 cm long, 30 cm high, and 10 cm wide) and labeled A, B, and C. The spontaneous alternation

task was conducted in 2 consecutive days – a training session on the first day and a retention test on the following day. The rat was positioned in the middle of the Y-maze and allowed to investigate the arms for 5 min. The consecutive entry into all three arms without entering the same arm twice in a row is recognized as an alternation. The right triplets of alternation are ABC, ACB, BAC, BCA, CAB, and CBA.[31] Spontaneous alternation % (SA%) was calculated using the formula:

$$SA\% = \frac{\text{Number of alternations}}{\text{Total number of entries } - 2} \times 100$$

Novel object recognition test (NORT)

This task is used to evaluate exploratory behavior and recognition memory in rodents. The experiment was performed in an open acrylic glass box (60 cm long, 60 cm wide, 40 cm high) in 2 consecutive days. The experimental protocol comprises of 3 phases: habituation, exploration (investigation), and testing. All rats were habituated in the test chamber for 5 minutes in the absence of any objects. Following that, the animals were placed into the test set up with two identical objects and allowed to investigate for 5 min. The memory trial was conducted 24 hours later. One of the items used in the learning session was replaced with a novel one and the animal was permitted to investigate them again for 5 minutes. The time spent by the rat exploring the novel (N) and familiar (F) object was recorded.[32] Recognition index (RI) was determined using the formula:

$$RI = \frac{N}{N + F} \times 100$$

where N is the novel object exploration time, and F is the familiar object exploration time

Sample collection and immunological assay

Pyrogen and endotoxin free collecting tubes were used. Blood samples were centrifuged (for 10 minutes) following clotting. The serum was carefully separated, aliquoted and frozen at −70°C. Serum levels of TNF-α, IL-1β, TGF-β1 and BDNF were measured using solid-phase ELISA. Absorbance was read at 450 nm with ELISA reader and recalculated as a concentration (pg/ml) using a standard curve. The sensitivity was as follows: TNF-α – 15 pg/ml; IL-1β – 4.4 pg/ml, TGF-β1 – 48 pg/ml, and BDNF – 18.8 pg/ml.

Statistical analysis

Statistical analysis was performed by IBM SPSS Statistics 19.0. All data were expressed as mean ± SEM. Data were analysed by one-way ANOVA, followed by LSD (least significant difference) post hoc test for comparisons between the groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effects of cholecalciferol on learning and memory in rats with LPS-induced model of inflammation

Step-through inhibitory "passive" avoidance test

The LPS control markedly decreased the latency time in comparison to olive oil control during the two memory tests ($p<0.05$). Both experimental groups (treated with cholecalciferol at doses 500 and 1000 IU/kg bw) significantly increased the latency during the training days ($p<0.05$) as well as during the short-term ($p<0.05$ and $p<0.01$, respectively) and long-term memory tests ($p<0.05$ and $p<0.01$, respectively) when compared to the LPS control (**Fig. 1**).

T-maze

The animals from the LPS treated control group showed significant decrease in working memory index in comparison to the control ($p<0.05$). Both cholecalciferol doses (500 and 1000 IU/kg bw) markedly increased this index when compared to the LPS control ($p<0.01$; $p<0.05$, resp.). Moreover, the lower dose of vitamin D_3 showed significant elevation in the observed parameter even against the olive oil treated animals ($p<0.05$) (**Fig. 2**).

Y-maze

The LPS-challenged rats showed significant decrease of the SA% on the retention test in comparison to the control ($p<0.05$). Vitamin D_3 in both studied doses (500 and 1000 IU/kg bw) significantly increased the SA% on day 1 ($p<0.05$ for both doses) and day 2 ($p<0.001$; $p<0.05$ resp.) when compared to LPS control. Furthermore, the lower dose of cholecalciferol increased the SA% on both experimental days in comparison to olive oil control ($p<0.05$ and $p<0.01$, resp.) (**Fig. 3**).

NORT

Animals from the LPS control group demonstrated significant decrease in recognition index in comparison to olive oil control ($p<0.05$). Rats treated with LPS and vitamin D_3 in doses of 500 and 1000 IU/kg bw significantly increased the recognition index when compared to olive oil ($p<0.001$) and LPS ($p<0.001$) control (**Fig. 4**).

Effects of cholecalciferol on serum levels of TNF-α, IL-1β, TGF-β1 and BDNF in rats with LPS-induced model of inflammation

TNF-α

The animals injected with LPS demonstrated significantly increased serum levels of TNF-α in comparison to the control ($p<0.001$). Both experimental groups treated with
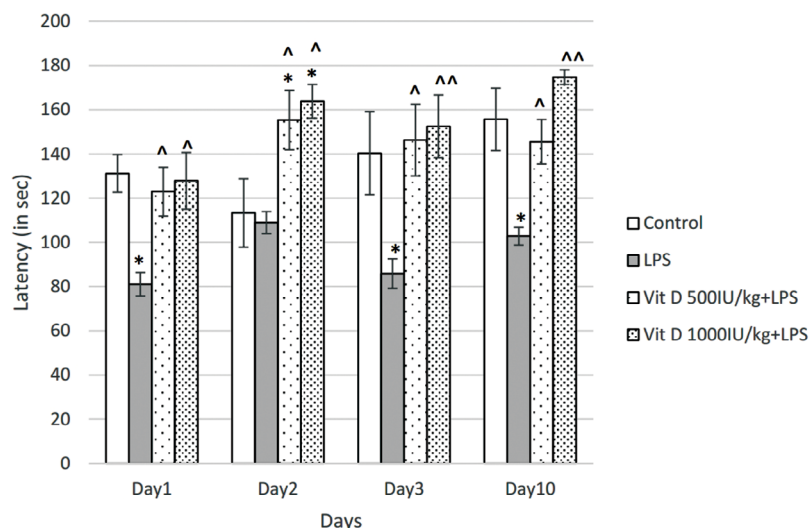
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Figure 1. Effects of cholecalciferol on step-through inhibitory "passive" avoidance test in rats with LPS-induced model of inflammation. *$p<0.05$ compared to control; ^$p<0.05$ compared to LPS control; ^^$p<0.01$ compared to LPS control.
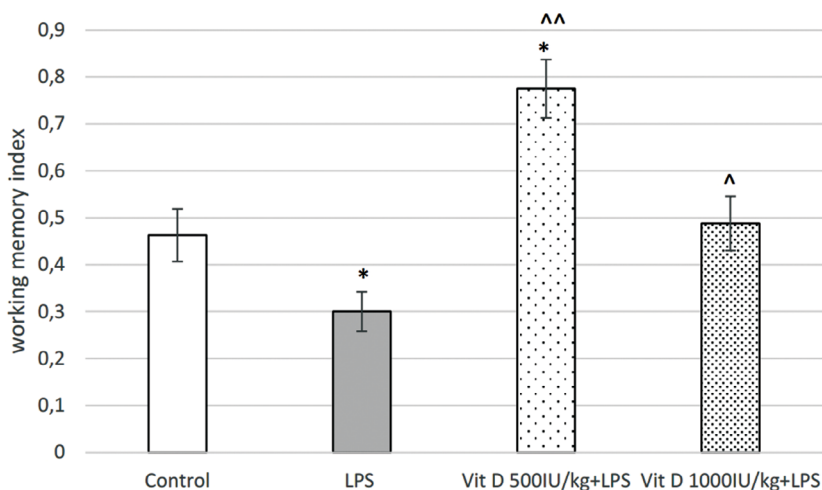

Figure 2. Effects of cholecalciferol on T-maze task in rats with LPS-induced model of inflammation. *$p<0.05$ compared to control; ^$p<0.05$ compared to LPS control; ^^$p<0.01$ compared to LPS control.
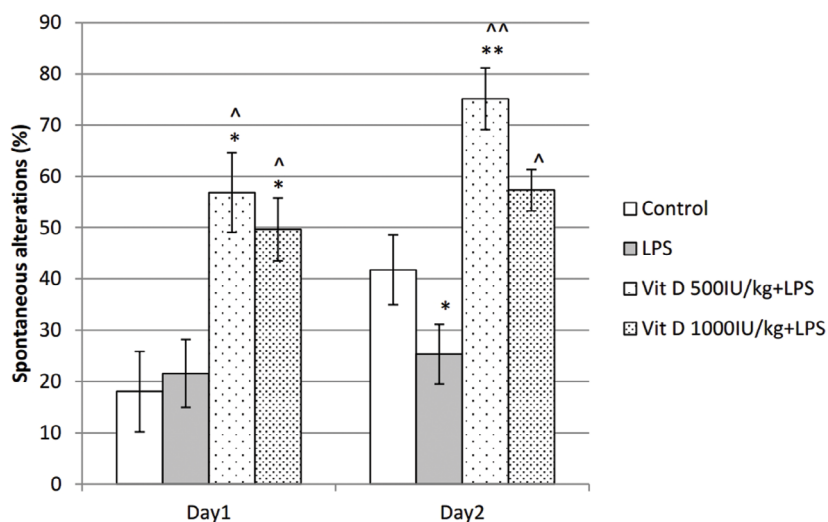

Figure 3. Effects of cholecalciferol in the Y-maze task in rats with LPS-induced model of inflammation. *$p<0.05$ compared to control; **$p<0.01$ compared to control; ^$p<0.05$ compared to LPS control; ^^$p<0.001$ compared to LPS control.
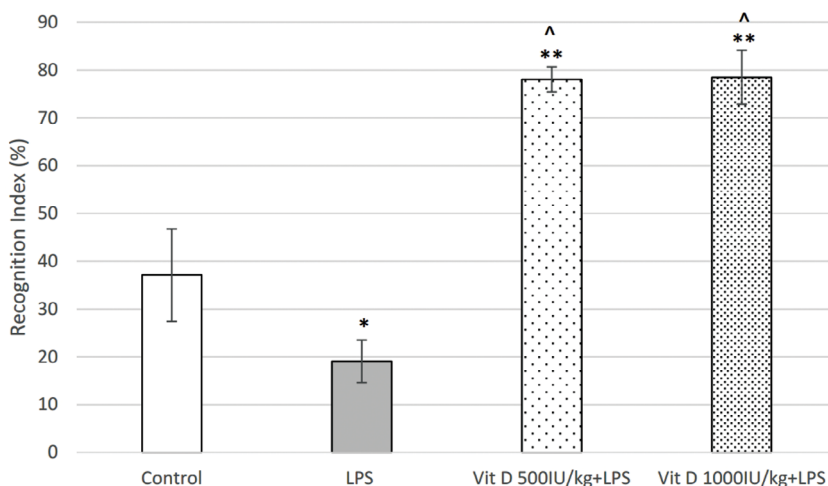
Figure 4. Effects of cholecalciferol on recognition index in rats with LPS-induced model of inflammation. *$p<0.05$ compared to control; **$p<0.001$ compared to control; ^$p<0.001$ compared to LPS control.

cholecalciferol markedly lowered TNF-α levels when compared to the LPS-challenged rats ($p<0.001$ and $p<0.01$, resp.) **(Table 1)**.

Table 1. Effects of cholecalciferol on TNF-α serum levels following LPS-induced inflammation

Groups	TNF-α (pg/ml) mean±SEM
Control	0.1±0
LPS	39.722±13.9*
LPS + vitamin D 500 IU/kg bw	2.25±0.8^^
LPS + vitamin D 1000 IU/kg bw	5.95±1.05^

*$p<0.001$ compared to control; ^$p<0.01$ compared to LPS control; ^^$p<0.001$ compared to LPS control.

IL-1β

The animals injected with LPS significantly increased IL-1β serum levels compared to controls ($p<0.05$). The group treated with vitamin D_3 at a dose of 500 IU/kg markedly lowered IL-1β concentration in comparison to LPS-challenged group ($p<0.05$). The higher dose of vitamin D_3 also decreased IL-1β serum levels but the difference did not reach statistical significance **(Fig. 5A)**.

BDNF

The LPS-challenged rats markedly decreased the serum concentration of BDNF in comparison with the control animals ($p<0.01$). Both experimental groups with cholecalciferol (500 and 1000 IU/kg) significantly elevated the BDNF serum levels when compared to the LPS control ($p<0.05$) **(Fig. 5B)**.

TGF-β1

The animals from the LPS control significantly reduced the TGF-β1 serum levels when compared to those treated with olive oil ($p<0.05$). The rats treated with 1000 IU/kg bw vitamin D_3 significantly elevated TGF-β1 serum concentration in comparison to the LPS control ($p>0.05$) **(Fig. 5C)**.

DISCUSSION

Vitamin D deficiency is considered a risk factor for memory decline in patients with neurodegenerative disorders. This vitamin has a well-established neuroprotective effect and plays an immunoregulatory role. Neuro- and systemic inflammation correlate with cognitive impairment, including memory loss. The present study investigated the role of cholecalciferol (vitamin D_3) supplementation on memory and serum levels of pro-inflammatory cytokines, TGF-β1,
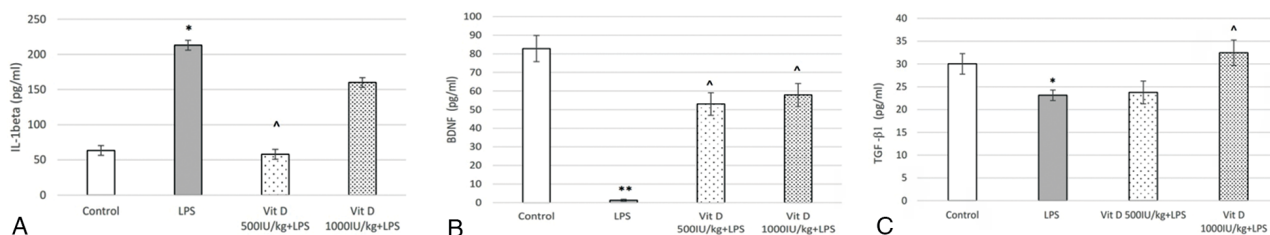

Figure 5. Effects of cholecalciferol on serum levels of IL-1β, BDNF and TGF-β1 in rats with LPS-induced model of inflammation. **A.** IL-1β; **B.** BDNF; **C.** TGF-β1 ; *$p<0.05$ compared to control; **$p<0.01$ compared to control; ^$p<0.05$ compared to LPS control.

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and BDNF in an animal model of neurodegeneration and neuroinflammation. The obtained results indicate that vitamin D_3 improves spatial working and episodic memory. The neuroprotective effect of this neurosteroid is thought to be related to its immunomodulatory, antioxidant properties and ability to increase expression of neurotrophic factors. The observed memory improving effect of cholecalciferol in the present study might be explained with its ability to regulate the production of pro-, anti-inflammatory cytokines, and BDNF.

In all memory tasks, the animals from the LPS control group showed significant decline in memory functions. Peripheral inflammation induced by systemic administration of LPS affects the brain and contributes to the development of neuroinflammation. LPS activates microglia, induces expression of pro-inflammatory cytokines in different brain regions, causes oxidative stress and neuronal loss.[25] Large number of studies illustrated that LPS-induced neuroinflammation is an appropriate model for studying cognitive decline in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. A recent experimental study[33] showed that both systemic and intracerebroventricular administration of LPS causes learning and memory impairment. The results from the current study are consistent with previous studies illustrating the effect of LPS on memory functions.

We further investigated the effect of cholecalciferol on learning and memory in rats with LPS-induced inflammatory response. In animals treated with both doses of vitamin D_3, we registered a significant improvement in the short- and long-term memories in step-through inhibitory avoidance (IA) task. Cholecalciferol treated rats were able to remember the foot shock received in the learning session and spent more time in the light chamber during the short- and long-term memory testing (at days 3 and 10). IA memory depends on the morphological and functional integrity of the hippocampal CA1, entorhinal and posterior parietal cortex and is modulated by the amygdala and other brain areas.[34] Based on the results from step-down passive avoidance test, we may speculate that the hippocampus plays an important role in the observed memory enhancing effect of cholecalciferol.

NORT is used to study episodic memory. The test is based on the innate curiosity of rodents and their instinct to explore a new item. The improvement in memory functions is established by extending the time spent in exploring the new object. The brain structure involved in the encoding and consolidation of object memory is the hippocampus although the perirhinal cortex may also participate.[35] A recent study[36] showed that vitamin D improves recognition memory in rats on chronic high fat diet. As IL-1β has been found to cause memory decline in NORT[37] and the present study demonstrated decreased serum levels of this cytokine, we can speculate that the immunomodulatory role of vitamin D may contribute to its improving effect on episodic memory.

Vitamin D receptors are present and functional in hippocampal neurons and glial cells. Immunocytochemical analysis shows that these receptors are localized in the dentate gyrus, CA1, CA2, and CA3 subfields.[38] Behavioral, neuroanatomical, neurochemical, and in vitro studies showed that vitamin D is important for hippocampal developing and functioning.[39] The memory improving effect of calciferol in hippocampal-dependent tests could be explained with its neuroprotective effects in this brain structure demonstrated in numerous preclinical studies. In aging rats, vitamin D improves hippocampal-dependent memory and up-regulates genes and functional pathways important for the normal synaptic function and calcium regulation in the hippocampus.[9] Guo et al.[40] showed that 1,25-dihydroxyvitamin D_3 decreases the number of pathological pyramidal neurons and improves hippocampal metabolism in rats with streptozotocin-induced diabetes. In this model vitamin D supplementation reduces overexpression of neuronal nitric oxide synthase (nNOS) and amyloid precursor protein, restores VDR, and down-regulates overactivation of endoplasmic reticulum stress in the hippocampus. Vitamin D has an antiapoptotic effect on hippocampal cells. Recent in vivo studies[41,42] demonstrated that this vitamin reduces the expression of pro-apoptotic proteins caspase-3 and Bax, and causes reduction of the anti-apoptotic protein Bcl-2. The regulation of oxidative stress by vitamin D is also implicated in its neuroprotective effect. Studies on rats with experimental model of Alzheimer's disease indicate that vitamin D has an antioxidant effect determined by its ability to increase total antioxidant capacity, total thiol groups, and reduce lipid peroxidation and DNA damage in both serum and hippocampus.[43] Experimental studies found that vitamin D increases expression of the NRI subunit of the N-Methyl-D-Aspartate (NMDA) glutamate receptor in the hippocampus of rats with fetal growth retardation.[13]

Y- and T-maze are behavioral tests for assessing spatial working memory. In T-maze, behavioral responses seem to be encoded by neurons of the medial prefrontal cortex.[44] The results from the current study showed that both doses of cholecalciferol improved spatial working memory in this task. The beneficial effect of cholecalciferol on LPS-induced memory impairment in T-maze assumes that the prefrontal cortex might also be implicated in the observed outcome. The neuroprotective effect of vitamin D on brain cortex was demonstrated in in vivo and in vitro studies. Chronic treatment with vitamin D_3 protects rat primary cortical neurons cultures against glutamate induced cytotoxicity.[45] In rats on high fat diet, vitamin D_3 supplementation improves cholinergic mediation, which plays an important role in memory processing, in the prefrontal cortex.[36]

In Y-maze task spatial working memory is assessed by recording the % SA. Two types of SA behavior are used to investigate memory in experimental animals – two-trial (forced-choice procedure) and continuous (free-trial) alternation. In our study, we used the second one. Rats were allowed free access to the three arms of the maze. Animals with intact memory would remember which arm they had visited previously and would enter the unvisited arm of the

maze. An alternation represents consecutive entries into three different arms of the Y-maze.[46] In this task the memory improving effect of cholecalciferol was evaluated by calculating the % SA. Both doses of vitamin D significantly increased the % SA compared with the LPS control. Continuous alternation requires normal functioning of the prefrontal cortex[47] but the hippocampus is also involved.[48] The results from the T- and Y-maze in the present study indicate that not only the hippocampus but also the prefrontal cortex may be involved in the memory improving effect of cholecalciferol in the settings of peripheral and neuro-inflammation.

High levels of pro-inflammatory cytokines in the brain are involved in the pathogenesis of memory decline. Systemic inflammatory response increases production of inflammatory mediators in the CNS and markedly affects brain functions, including memory.[25] A recent study[49] demonstrated that the memory improving effect of vitamin D₃ in LPS-induced inflammation is due to its ability to suppress inflammation and oxidative stress in the hippocampus. In order to link the observed beneficial effect of vitamin D on memory with its immunomodulatory action, we additionally examined the effect of cholecalciferol on TNF-α, IL-1β and TGF-β1 serum levels.

TNF-α is produced by both neurons and glial cells but in the pathogenesis of neuroinflammation, microglia play the most important role.[50] Recent in vitro study[51] demonstrates that microglia is the major source of TNF-α and its production is massively increased by LPS. TNF-α produced by peripheral immune cells may also contribute to the development of inflammation in the CNS as this cytokine can penetrate the brain by a saturable transport system present at the blood-brain barrier.[52] Increased TNF-α levels in the periphery and brain are associated with memory impairment. An animal study[53] has shown that acute stress increases microglial production of TNF-α in the hippocampus leading to impaired working memory, which was alleviated by the administration of the TNF-α inhibitor etanercept. Other studies demonstrated that increased serum TNF-α stimulates the peripheral inflammatory cascade resulting in cognitive decline.[54] Vitamin D plays an immunomodulatory role in the body. Previous study[55] showed that in an animal model of autoimmune encephalomyelitis, 1,25-dihydroxyvitamin D3 decreases RNA expression and production of IFN-γ, TNF-α, IL-6, and IL-17 in spleen and lymph node cells. Experimental data demonstrate that cholecalciferol inhibits TNF-α production not only in the immune system but also in the brain.[56]

Excessive levels of IL-1β have been shown to inhibit long-term potentiation (LTP) in the hippocampus.[57] LTP is based on synaptic plasticity and is considered as the cellular substrate of learning and memory.[58] In our study, LPS administration significantly increased serum IL-1β and TNF-α concentrations. Previous studies demonstrated that peripheral inflammation is linked with neuroinflammation and increased expression of IL-1β in the hippocampus. In aging rats peripheral infection causes

pronounced and long-lasting increase in hippocampal IL-1β levels associated with deficits in hippocampal memory consolidation.[59] Li et al.[37] found that systemic administration of LPS in mice increases hippocampal IL-1β and TNF-α levels, induces oxidative stress, decreases BDNF levels and causes cognitive dysfunction. These effects were prevented in mice with lentivirus-induced IL-1β knockdown in the hippocampus. We can assume that increased serum IL-1β and TNF-α in the current research is associated with elevated hippocampal expression of these molecules. The cellular mechanisms involved in the inhibition of memory consolidation by IL-1β include stimulation of hippocampal p38 MAPK, inhibition of glutamate release, delay in EPK activation, and decreased BDNF expression.[60] The decreased memory function in LPS treated control could therefore be explained with increased hippocampal IL-1β and TNF-α levels.

Animal studies demonstrate that vitamin D decreases IL-1β expression in the brain. Farhangi et al.[61] showed that this vitamin reduces IL-1β in the hypothalamus of rats receiving a normal diet whereas reduction was not significant in animals on high fat diet. In aging rats, vitamin D₃ treatment decreases age-related microglial activation and IL-1β levels in the hippocampus.[62] A recent study[63] found that vitamin D reduces TNF-α and IL-1β levels probably by inhibiting NF-κB expression in brain homogenates of mice with D-galactose induced memory impairment. In our study cholecalciferol in a dose of 500 UI/kg bw significantly decreased IL-1β and TNF-α serum concentrations and improved memory consolidation. Peripherally produced cytokines induce production of pro-inflammatory mediators from microglia and might be actively transported through the blood-brain barrier.[64] Vitamin D-induced decrease in serum IL-1β and TNF-α levels would interrupt the link between systemic and neuroinflammation. Thus, we can speculate that the memory improving effect of cholecalciferol in the current study is at least partially associated with decreased IL-1β and TNF-α production.

Recent studies showed that vitamin D interacts with the expression of TGF-β1. However, data are controversial. Calvello et al.[65] has demonstrated that the administration of this vitamin increases brain TGF-β levels in an animal model of Parkinson's disease. The protective effect of vitamin D against an experimental autoimmune encephalomyelitis (a preclinical model of multiple sclerosis) also seems to be at least partially related with increased TGF-β expression, including in the lymph nodes.[66] Another study[67] found that vitamin D suppresses hippocampal and spleen expression of TGF-β in mice with cognitive decline and immune system activation due to surgical trauma. The results of the present study showed that in rats with LPS-induced inflammation pretreatment with cholecalciferol increases serum TGF-β1 levels. Based on the data about its neuroprotective effect and the fact that this growth factor may penetrate disrupted blood-brain barrier[68] we can speculate that the observed memory enhancing effect of cholecalciferol may also be explained with its effect on TGF-β1.

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Clinical studies show that increased serum TGF-β levels could also be associated with peripheral beneficial effects. Rasa et al.[69] found that vitamin D induced elevation in TGF-β1 serum levels might be responsible for the antiatherosclerotic effect of this vitamin. Thus, the up-regulating effect of cholecalciferol on TGF-β1 is probably responsible for positive outcomes beyond the memory improving effect of this neurosteroid and hormone.

BDNF promotes the growth and survival of a number of neurons, including cortical and hippocampal neurons.[70] It is a member of the family of neurotrophins and exerts its effects through binding to tyrosine protein kinase receptor B (trkB). BDNF and its receptor are expressed in the hippocampus where they play an important role in neuronal plasticity, including LTP.[71] Recent preclinical studies showed that vitamin D supplementation increases brain level of this neurotrophic factor in aged rats.[72] In rats with high-fat diet induced obesity, the studied vitamin increases hippocampal BDNF levels and improves memory functions in Morris water maze.[73] In the present study, we detected elevated BDNF serum levels in vitamin D treated rats with LPS-induced model of neuroinflammation. A previous study[74] found that there is strong correlation between BDNF serum and hippocampal levels in rats. We can suggest that cholecalciferol in the studied doses would also increase BDNF production in the hippocampus. Thus, the observed memory improving effect of vitamin D in the hippocampal dependent memory tasks might be due to elevated BDNF synthesis. Since IL-1β inhibits the expression and action of this neurotrophic factor[19], we can speculate that decreasing IL-1β is one of the possible mechanisms by which vitamin D stimulates BDNF production.

CONCLUSIONS

Cholecalciferol improves spatial working and episodic memory in LPS induced model of systemic and neuroinflammation. The observed effect can at least partially be explained with its effect on systemic inflammatory response as it decreases serum levels of pro-inflammatory TNF-α and IL-1β, and upregulates TGF-β1 and BDNF production.

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Competing interests

The authors of this manuscript have declared that no competing interests exist.

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Витамин D₃ оказывает иммуномодулирующее действие и улучшает память у крыс с липополисахарид-индуцированным воспалением

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Резюме

Введение: Витамин D является жирорастворимым секостероидом, основной функцией которого является регуляция кальциево-фосфатного гомеостаза и поддержание целостности и минерализации костей. Недавно были признаны плеотропные эффекты этого витамина, включая иммуномодулирующую роль и участие в нормальном развитии и функционировании мозга.

Цель: Целью настоящего исследования было изучение влияния холекальциферола на сывороточные маркеры воспаления и функции памяти в липополисахаридной (ЛПС) модели воспаления.

Материалы и методы: Самцов крыс Wistar случайным образом разделили на 4 группы (n=8): контрольная группа, контрольная группа ЛПС, группа ЛПС + холекальциферол (витамин D₃) 500 UI и группа 1000 IU / kg массы тела. Для оценки функций памяти были проведены тест пассивного избегания (Step-down passive avoidance test), тест распознавания новых объектов (novel object recognition test NORT), Y- и Т-лабиринт. Регистрировали латентность, индекс распознавания (RI), % спонтанных изменений (SA) и индекс рабочей памяти. Уровни фактора некроза опухоли-альфа (TNF-α), IL-1β, трансформирующего фактора роста-β1 (TGF-β1) и нейротрофического фактора головного мозга (BDNF) измеряли в сыворотке с помощью ELISA.

Результаты: Введение ЛПС вызвало значительное ухудшение функций памяти во всех задачах на память. Лечение холекальциферолом вызывало достоверное увеличение % SA, RI и индекса рабочей памяти. В ступенчатом тесте пассивного избегания группы, получавшие холекальциферол, показали статистически значимое увеличение латентного периода в тесте на долговременную память. У крыс, получавших витамин D₃, наблюдалось снижение уровней TNF-α и IL-1β в сыворотке крови, тогда как концентрация TGF-β1 и BDNF увеличивалась.

Заключение: Холекальциферол улучшает пространственную рабочую и эпизодическую память, что хотя бы частично можно объяснить его влиянием на системный воспалительный ответ, тесно связанный с развитием нейровоспаления.

Ключевые слова

холекальциферол, цитокины, воспаление, липополисахарид, память