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VITAMIN D_3 AFFECTS GLUCOCORTICOID-SENSITIVE RECEPTORS AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN PREDNISOLONE-INDUCED NEUROTOXICITY

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Significant attention is currently paid to the study of glucocorticoid (GC)-induced structural and functional changes in the central nervous system (CNS). GCs are widely used as effective anti-inflammatory and immunosuppressive drugs. Still, the balance between the anti-inflammatory and pro-inflammatory effects of glucocorticoids in the CNS can be disrupted depending on the dosage, duration of GC supplementation, GR (glucocorticoid receptor)/MR (mineralocorticoid receptor) ratio, and genetic variability of these receptors [1]. Various sources of evidence indicate that GCs and their receptors interact with one of the major neurotrophins in the CNS, the brain-derived neurotrophic factor (BDNF), influencing important aspects of neuronal cell physiology such as cell viability, memory formation, behavioral responses, and adaptation to stressful stimuli [2]. In addition, vitamin D_3 (D_3) deficiency and impaired signaling via D_3 receptor (VDR) were previously reported to be essential contributing factors in the development of GC-induced adversary effects in the CNS [3]. However, the molecular mechanisms of D_3 involvement in the regulation of steroid receptors interplay and BDNF expression have not been sufficiently studied.

Aim. To explore how long-term GC administration affects the GR/MR ratio and BDNF protein level, as well as their relationship with the D_3 status of experimental animals.

Methods. Female Wistar rats received prednisolone at a dose of 5 mg/kg body weight with or without D_3 (1000 IU/kg body weight) for 30 days. Serum 25-hydroxyvitamin D_3 ($25D_3$) and brain tissue BDNF levels were measured by ELISA. We used western blotting to determine GRα/β and MR protein levels. The number of astrocytes in histological sections of the cerebral cortex and hippocampus CA1-CA3 regions was assessed by immunofluorescent labeling of the macroglial marker protein GFAP (glial fibrillary acidic protein). Data were statistically analyzed using one-way ANOVA followed by *Tukey's* test. The significant level was set at $P < 0.05$.

Results and Discussion. Using an experimental model of GC-induced neurotoxicity, we first examined the degree of bioavailability of D_3 (based on the level of $25D_3$ in serum), as well as the relationship between the pool of circulating $25D_3$ and its level in rat brain tissue, Table. A significant 2.52-fold reduction of $25D_3$ in the blood serum was revealed that correlated with a 2.12-fold decrease in the content of this metabolite in the nervous tissue compared to control animals. Therapeutic administration of D_3 against the background of prednisolone led to a rise in the level of $25D_3$ in the blood serum and brain by 1.94 and 1.43 times, respectively.

GCs are known to act specifically through GRα/β but are also able to bind to MR with less affinity. Literature data suggest that activation of MR can effectively prevent GR-induced neuronal loss, indicating the involvement of MR in the neuroprotective effects of GC and the critical role of tissue distribution and balance of these two types of nuclear receptor proteins for cell survival [1]. The revealed D_3-deficient status of animals under GC loading was accompanied by an increase in the content of GRα/β protein in the brain by 1.80 times, while the level of MR protein decreased by 1.28 times compared to the control. D_3 treatment reduced GR levels in the brain without significantly affecting MR. The GR/MR ratio raised to 2.5 with prednisolone and decreased to 1.5 with D_3, indicative of the

Vitamin D3 status, brain tissue BDNF level, and GR/MR ratio

Control group	Prednisolone group	Prednisolone + D3 group
Serum $25D_3$, nmol/l		
108.5 ± 8.3	42.9 ± 5.0*	83.6 ± 8.1#
Brain tissue $25D_3$, ng/g		
19.05 ± 1.32	8.98± 0.72*	12.90 ± 0.90#
Brain tissue BDNF, ng/g		
3.19 ± 0.27	4.56 ± 0.40*	3.73 ± 0.31#
GR/MR ratio		
1.0± 0.08	2.5 ± 0.19*	1.5 ± 0.11#

Note: All data are presented as mean ± SEM, $n = 6$; * — $P \le 0.05$ denotes significance compared with control, # — $P \le 0.05$ denotes significance compared with prednisolone action.

cytotoxic mechanisms of prednisolone long-term action and the significant effectiveness of D_3 in the correction of GC-induced disorders in the CNS (Table).

Given the close association between GC and BDNF signaling, we next examined GC-induced alterations of brain BDNF expression. Our data indicate that long-term exposure of rats to prednisolone resulted in a marked (42%) increase in brain BDNF protein level compared to control, Table. Taken together, these findings suggest that BDNF might be involved in initial stress response, playing a compensatory role by preventing oxidative damage to cell structures and low-grade inflammation as well as depressive-like behavior identified in our previous work [3]. We demonstrated the ability of D_3 to partially normalize brain BDNF protein levels compared to prednisolone-treated animals.

Finally, we focused on a particular type of glial cells, astrocytes, which were found to produce and secrete BDNF, while simultaneously expressing BDNF receptor — TrkB [4]. In addition, astrocytes have characteristics of immune cells as they are activated upon local immune stimulation, thereby providing a suitable substrate for GC-mediated immune responses and playing an important role in various neuropathological conditions. Prednisolone increased the number of astrocytes in rats' cerebral cortex and hippocampus CA1-CA3 regions by 30 and 38%, respectively. D_3 treatment partially corrected the number of astrocytes in these brain regions.

Conclusions. Thus, we established an increase in the GR/MR ratio due to an upregulation of GRα/β and downregulation of MR levels in the nervous tissue that correlated with the manifestations of neuropathological changes induced by long-term exposure to prednisolone. In accordance with the protective paradigm, an elevation of BDNF synthesis was found, most likely associated with an increase in the number of astroglial cells in different sections of the rat brain. These abnormalities were shown to be accompanied by D_3 deficiency in animals. Supplementation of D_3 completely or partially reversed the alterations caused by long-term administration of prednisolone. Based on the identified positive effects of D_3 on the CNS, its practical usefulness in the complex treatment of neurological and cognitive disorders associated with GC-based therapeutics can be envisaged.

Key words: Glucocorticoid-induced neurotoxicity, prednisolone, vitamin D3, brain-derived neurotrophic factor, steroid receptors, astrocytes.

Authors' contribution. DIY — preparing protein lysates, BDNF measurement and presentation of the results, OOL — performing western blots, AVK — detecting $25(OH)D_3$ levels, IOS — conceptualization and supervision, macroglia labeling, data analysis. All authors have read and agreed to the published version of the abstract.

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