Acta Neurol Scand 2010: 122 (Suppl. 190): 88–92

ACTA NEUROLOGICA SCANDINAVICA

Assessing vitamin D in the central nervous system

Holmøy T, Moen SM. Assessing vitamin D in the central nervous system. Acta Neurol Scand: 2010: 122 (Suppl. 190): 88–92.

Epidemiological and experimental evidence suggest that vitamin D deficiency is a risk factor for multiple sclerosis and other autoimmune diseases. The activated form of vitamin D exerts several immunomodulating properties *in vitro* and *in vivo*, that could contribute to explain the association with multiple sclerosis. Hypovitaminosis D is also associated with several other neurological diseases that is less likely mediated by dysregulated immune responses, including Parkinson's disease and Alzheimer's disease, schizophrenia and affective disorders, suggesting a more diverse role for vitamin D in the maintenance of brain health. Accordingly, both the vitamin D receptor and the enzymes necessary to synthesize bioactive 1,25-dihydroxyvitamin D are expressed in the brain, and hypovitaminosis D is associated with abnormal development and function of the brain. We here review current knowledge on the intrathecal vitamin D homeostasis in heath and disease, highlighting the need to assess vitamin D in the intrathecal compartment.

T. Holmøy[1,2], S. M. Moen[1,2]

[1]Department of Neurology, Oslo University Hospital Ullevål, Oslo, Norway; [2]Faculty of Medicine, University of Oslo, Oslo, Norway

Conflicts of interest: TH has received consultant or speakers honorarium from Biogen, Sanofi Aventis, Merck Serono, and Novartis. SMM declares no conflict of interest.

Key words: cerebrospinal fluid; multiple sclerosis; vitamin D

Trygve Holmøy, Department of Neurology, Oslo University Hospital Ullevål, Oslo, Norway
Tel.: +47 22118080
Fax: +4723073710
e-mail: trygve.holmoy@rr-research.no

Introduction

The roots of the vitamin D discovery date back to the epidemics of rickets during the industrial revolution (1). In 1918 Sir Edward Mellanby at the University of Sheffield discovered that rickets was caused by nutritional deficiency. It was soon recognized that cod liver oil cured rickets. The German pediatrician Kurt Huldschinsky had no access to cod liver oil for his impoverished patients. He was, however, aware that rickets rarely occurred in children who got a lot of sun, and he therefore started to treat rickets with a quartz lamp and proved the effect in 1919 (2). 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the bioactive metabolite, was isolated and named vitamin D in 1922. In 1924 three different groups co-discovered that sunlight is a source of vitamin D (3–5).

During the last decades, it has become evident that vitamin D is involved in a multitude of biological processes beyond bone and calcium homeostasis (6). The beneficial effects of sunlight for non-skeletal aspects of human health were recognized before the discovery of vitamin D. In 1903, Niels Finsen received the Nobel Prize in Medicine for using ultraviolet light to treat tuberculosis (7). The molecular mechanisms for this have recently been explored, and seem to involve upregulation of endogenous antimicrobial peptides such as cathedrin and definsins in macrophages, neutrophils and epithelial cells (8). The antiproliferative effect of vitamin D was early shown to involve lymphocytes and other immune cells (9). In general, such non-skeletal effects of vitamin D can be divided into three overlapping categories: (i) regulation of immune function, (ii) regulation of cell proliferation, and (iii) regulation of secretion of hormones, growth factors and cytokines. Of particular interest to neurologists, vitamin D has been shown to play an important role in brain development, and has also been suggested as an environmental risk factor and as a potential treatment in a variety of neurological diseases, including multiple sclerosis (MS), Parkinson's disease and Alzheimer's disease, schizophrenia, epilepsy and affective disorders (10).

Given the emerging interest in vitamin D in brain health, there has been a remarkable lack of data on intrathecal vitamin D homeostasis in humans. Very little is thus known about vitamin D homeostasis in the human brain during health and disease. The aim of this non-exhaustive review

is to highlight the need to assess vitamin D in the intrathecal compartment.

Physiology of vitamin D

Sunlight, diet, and dietary supplements are sources of vitamin D for humans. There are two major forms of vitamin D, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Both forms can be found in foods, whereas only vitamin D_3 is produced in the skin upon exposure to UVB radiation followed by thermally induced isomerization. Vitamin D_3 then enters the circulation bound to vitamin D binding protein, and is subsequently hydroxylated to 25-hydroxyvitamin D (25(OH)D) and further to bioactive $1,25(OH)_2D$. It was formerly believed that synthesis of $1,25(OH)_2D$ is restricted to the liver and kidneys. It is now recognized that several tissues, including the brain and several different immune cells, express the vitamin D 1α-hydroxylase (CYP27B1) that is the limiting enzyme for synthesis of $1,25(OH)_2D$ (11–13). Both the brain and the immune system may therefore respond to $1,25(OH)_2D$ in either an autocrine or paracrine fashion.

Most biological effects of $1,25(OH)_2D$ start with binding to the nuclear vitamin D receptor, which subsequently heterodimerizes with the retinoic acid receptor. This complex of vitamin A and D and their receptors binds to particular genomic sequences named vitamin D response elements, which regulate the expression of several hundred or maybe thousand different genes (14, 15). In addition to the genomic effects mediated through the nuclear vitamin D receptor, $1,25(OH)_2D$ also binds membrane associated receptors which activate calcium channels and other signal transduction systems that operate too rapidly to involve altered gene expression (16).

Vitamin D and brain development

In addition to the enzymatic machinery needed to synthesize $1,25(OH)_2D$, the vitamin D receptor is also expressed throughout the human and rodent brain (12, 17). It has been shown *in vitro* that $1,25(OH)_2D$ regulates the expression of important growth factors in the brain such as nerve growth factor, glial cell line-derived growth factor, neurotrophin 3, and growth associated protein-43 (18–20). $1,25(OH)_2D$ also induces cell differentiation of embryonic hippocampal cells (21). These *in vitro* data have been supported by *in vivo* experiments showing that removal of vitamin D from the diet of pregnant rats leads to increased ventricle volume and decreased expression of nerve growth factor in their offspring (22, 23).

Vitamin D and neurological disease

Vitamin D has been most extensively studied in MS. The converging epidemiological and experimental data, both in the experimental autoimmune encephalomyelitis (EAE) model and in human studies have recently been reviewed (24). Vitamin D deficiency was first suggested to be a risk factor in MS by Goldberg, who hypothesized that MS resulted from an inadequate supply of vitamin D, magnesium, and calcium during remyelination and also reported decreased relapse rate after supplementation (25). The strongest epidemiological evidence comes from prospective studies showing that intake of vitamin supplements and also high 25(OH)D concentrations in serum are associated with low MS risk (26, 27). The vitamin D hypothesis is consistent with the correlation between MS risk and latitude that has been observed in several countries, and also with the increased MS risk for people born in May/June that has been observed in several countries including Sweden (28). Norway may be a remarkable exception to this general pattern, with a lower MS prevalence above the Arctic Circle. This paradox could possibly be explained by a high consumption of vitamin D rich fish and also by genetic factors, as the Sami population seems to be relatively protected against MS (29).

Several lines of evidence have pinpointed the immune system as the link between vitamin D and MS. Exposure of T helper cells from MS patients to $1,25(OH)_2D$ leads to a shift toward a more anti-inflammatory Th2-like and regulatory phenotype that is less likely to mediate brain inflammation (30, 31). $1,25(OH)_2D$ also upregulates the expression of the MS-associated HLA allele DRB1*1501 *in vitro* through interaction with a vitamin D response element in the DRB1*1501 promotor (32). HLA-DRB1*1501 is believed to participate in MS pathogenesis through presentation of immunogenic peptides to T helper cells, and this observation therefore supports an immunological basis for the vitamin D involvement in MS. This concept is also supported by *in vivo* studies showing that $1,25(OH)_2D$ prevents and blocks the development of EAE, which is driven by T helper cells (33). The protective effect is restricted to female mice, and depends on estrogen production by intact ovaries (34). A synergistic effect of estradiol and $1,25(OH)_2D$ was demonstrated in ovariectomized mice, as estradiol restored the EAE-resistance mediated by $1,25(OH)_2D$ and increased the

expression of the vitamin D receptor within the inflamed CNS (34). This is particularly interesting in light of the increasing female:male sex ratio in MS risk that has been observed in several countries including Norway (35), suggesting that females are particularly sensitive to emerging environmental risk factors.

Although the immune system is the main suspect linking vitamin D and MS, other possibilities that may be less easily studied should not yet be discarded. Alternative mechanisms may involve increased vulnerability of the CNS imposed by neonatal vitamin D deficiency, or that the vitamin D status interferes with remyelination in adult life as originally suggested by Goldberg (25). The first possibility is compatible with the month-of-birth effect on MS risk, suggesting that environmental risk factors may operate very early in life. The latter possibility is supported by *in vitro* studies showing that oligodendrocytes express the vitamin D receptor, and that $1,25(OH)_2D$ enhances the expression of nerve growth factor and its low affinity receptor p75 (36).

The widespread expression of the vitamin D receptor and the enzymatic machinery catalyzing the synthesis of $1,25(OH)_2D$ in the CNS has prompted the assumption that suboptimal levels of vitamin D plays a role also in other CNS diseases, including Parkinson's disease, epilepsy, depression, and schizophrenia (37). Accordingly, $1,25(OH)_2D$ seems to provide partial protection in a toxic model of Parkinson's disease induced by intraventricular administration of 6-hydroxydopamine (38), whereas vitamin D receptor knockout mice display increased seizure susceptibility and mortality in an experimental model of epilepsy (39). Not unexpectedly, low levels of 25(OH)D have been reported in patients with Parkinson's disease and depression (37). There is, however, an obvious need for prospective epidemiological studies showing that low levels of vitamin D actually predisposes to the disease, as the diseases themselves could lead to altered behavior with sun avoidance and dietary changes resulting in altered vitamin D levels.

Assessing vitamin D in the CSF

To enter the brain or CSF, vitamins must cross the blood–brain barrier in the cerebral capillaries or the blood–CSF barrier in the plexus choroideus. The tight junctions of the endothelium of the brain and the plexus choroideus restrict the passage of water-soluble substances. There are, however, several transport mechanisms for vitamins across the blood–brain barrier and blood–CSF barrier, and it has been estimated that $< 10\%$ of the transport of vitamin E, B1, B3, B6, pantothenic acid, and biotin can be accounted for by passive diffusion (40). The CSF is contiguous with the extracellular fluid of the brain and seems to serve as a conduit for transferring vitamins to different CNS regions. The CSF is therefore well suited for assessing the intrathecal vitamin status.

Vitamin D and its metabolites are lipophilic. They are, however, tightly bound to vitamin D binding protein, which has similar molecular size and charge as albumin. The concentration of vitamin D binding protein in the CSF, and hence the capacity to carry vitamin D, would therefore reflect the concentration of albumin unless there are specialized transport systems into the intrathecal compartment or intrathecal synthesis of vitamin D binding protein. Recent proteomic studies of human CSF have shown that the expression of vitamin D binding protein is either up or down-regulated in MS, Alzheimer's and Parkinson's diseases, and epilepsy (41–43), suggesting that the intrathecal homeostasis of vitamin D could be affected by these conditions. The findings are, however, not always consistent and need to be confirmed in studies that also asses differences in transport from serum.

Studies with vitamin D injection into mice suggest that there are limited transport of 25(OH)D and $1,25(OH)_2D$ to the brain (44, 45). Until 2009, only one published study had addressed vitamin D concentrations in the CSF of humans (46). Using high performance liquid chromatography, a well-established method for vitamin D measurement, Balabanova et al. detected surprisingly high CSF concentrations of 25(OH)D and $1,25(OH)_2D$. The concentrations reported were actually in the range of those commonly detected in the serum during winter and spring. This was surprising, given the similarities between vitamin D binding protein and albumin, and that the CSF:serum ratio of albumin is usually $> 1{:}100$. If true, these results clearly implicated that the intrathecal homeostasis of these vitamin D metabolites had to be governed by specific transport mechanisms.

To address this question, and also whether the intrathecal vitamin D homeostasis is distorted in MS, we used ultra performance liquid chromatography–mass spectrometry to measure 25(OH)D in the CSF and serum from 36 patients with relapsing remitting MS, 20 patients with other inflammatory diseases and 18 patients with non-inflammatory neurological diseases (47). Our results were clearly at odds with those previously published, as the concentrations of 25(OH)D were $< 1\%$ of those

recorded in serum, and also generally <1% of those previously published. They were, however, in line with those reported in a proceeding from 1980 that has later drifted into blivion (48). Importantly, 25(OH)D could not be detected in CSF from subjects with normal serum concentrations using standard radioiummunoassay or tandem liquid spectroscopy–mass chromatography, ruling out CSF concentrations in the range of those previously published (46).

Although the CSF concentration of 25(OH)D did not differ between MS patients and the control groups, MS patients displayed a substantially and significantly lower CSF:serum ratio than the control groups. It is not yet clear whether this reflects differences in blood–brain barrier integrity or other factors such as differences in the expression of vitamin D binding protein.

Conclusion

Further work needs to be carried out to understand the role for vitamin D in the brain in health and disease. From an epidemiological point of view, vitamin D should be assessed in prospective studies of Parkinson's disease and other non-MS diseases. In MS, the paradoxical north–south prevalence gradient in Norway needs to be explored. Does it really exist, and if so, can it be explained by differences in fish consumption or distribution of genetic risk factors? Moreover, is variation in genes involved in vitamin D metabolism associated with disease risk or severity in MS or other neurological diseases? Such genes have not (yet) been pinpointed by reliable whole genome screens. However, three MS patients have been reported with a very rare type of rickets caused by mutations in the CYP27B1 gene (49), suggesting that genes involved in vitamin D metabolism may be involved in MS. From an experimental point of view, other mechanisms than those related to the immune system should be addressed in animal models that are less dependent on inflammation than EAE. One interesting approach would be to study the effect of vitamin D on remyelination in the cuprizone model of demyelination. From a clinical point of view, the time has definitely come for large-scale clinical trials of high dose vitamin D supplementation in MS as add-on therapy to other immunomodulators.

There is also an obvious need for more knowledge on vitamin D in the CSF. Our study was focused on patients with relapsing remitting MS, whereas patients with clinically isolated syndromes are closer to disease onset and likely more informative about environmental risk factors. Moreover, vitamin D levels should be assessed in the CSF from patients with other neurological disorders where vitamin D might play a role. There is also a need to broaden the analytical repertoire. Given that the enzymes catalyzing the formation of $1,25(OH)_2D$ are expressed in the brain, we definitely need to develop sensitive assays that can accurately measure this biologically potent vitamin D metabolite in the CSF. This is important as increase or decrease in the intrathecal expression of 1α-hydroxylase could mediate pronounced differences in the concentration of $1,25(OH)_2D$ at the site of the disease process. The concentration of $1,25(OH)_2D$ is, however, generally much lower than that of 25(OH)D, and we have so far not been able to detect it in the CSF.

References

1. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest 2006;**116**:2062–72.
2. Haagensen CD, Lloyd WEB. A hundred years of medicine. Washington DC: Sheridan House, 1943. reprinted in 2000 by Beard Books.
3. Hess AF. On the induction of antirachitic prperties in rations by exposure to sunlight. Science 1924;**60**:269.
4. Hume EM, Smith HH. The effect of irradiation of the environment with ultra-violet light upon the growth and calcification of rats, fed on a diet deficient in fat-soluble vitamins. The Part played by irradiated sawdust. Biochem J 1924;**18**:1334–48.
5. Steenbock H. The induction of growth promoting and calcifying properties in a ration by exposure to light. Science 1924;**60**:224–5.
6. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009;**19**:73–8.
7. Mørner KAH. The Nobel Prize in Physiology or Medicine, 1903. Presentation Speech. http://nobelprize.org/nobel_prizes/medicine/laureates/1903/press.html.
8. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med 2010; Epub Feb 1 2010, PMID: 20119827.
9. Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-dihydroxyvitamin D3: a novel immunoregulatory hormone. Science 1984;**224**:1438–40.
10. Tuohimaa P, Keisala T, Minasyan A, Cachat J, Kalueff A. Vitamin D, nervous system and aging. Psychoneuroendocrinology 2009;**34**(Suppl. 1):278–86.
11. Neveu I, Naveilhan P, Menaa C, Wion D, Brachet P, Garabeidan M. Synthesis of 1,25-dihydroxyvitamin D3 by rat brain macrophages in vitro. J Neurosci Res 1994;**38**:214–20.
12. Eyles DW, Smith S, Kinobe R, Hewison M, Mcgrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat 2005;**29**:21–30.
13. Monakawa T, Hayashi M, Saruta T. Identification of 25-hydroxyvitamin D3 1alpha-hydroxylase gene expression in macrophages. Kidney Int 2000;**58**:559–68.
14. Bouillon R, Carmeliet G, Verlinden L et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008;**29**:726–76.

15. Wang TT, Tavera-Mendoza LE, Laperriere D et al. Large-scale in silico and microarray-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol 2005;**19**:2685–95.
16. Khanal RC, Peters TM, Smith NM, Nemere I. Membrane receptor-initiated signaling in 1,25(OH)2D3-stimulated calcium uptake in intestinal epithelial cells. J Cell Biochem 2008;**105**:1109–16.
17. Stumpf WE, Sar M, Clark SA, Deluca HF. Brain target sites for 1,25-dihydroxyvitamin D3. Science 1982;**215**:1403–5.
18. Naveilhan P, Neveu I, Wion D, Brachet P. 1,25-Dihydroxyvitamin D3, an inducer of glial cell line-derived neurotrophic factor. Neuroreport 1996;**7**:2171–75.
19. Wion D, Macgrogan D, Neveu I, Jehan F, Hougatte R, Brachet P. 1,25-Dihydroxyvitamin D3 is a potent inducer of nerve growth factor synthesis. J Neurosci Res 1991;**28**:110–4.
20. Taniura H, Ito M, Sanada N et al. Chronic vitamin D3 treatment protects against neurotoxicity by glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons. J Neurosci Res 2006;**83**:1179–89.
21. Marini F, Bartoccini E, Cascianelli G et al. Effect of 1alpha,25-dihydroxyvitamin D3 in embryonic hippocampal cells. Hippocampus 2009; Epub Feb 14 2009, PMID: 19603526.
22. Eyles DW, Feron F, Cui X et al. Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology 2009;**34**(Suppl. 1):247–57.
23. Feron F, Burne TH, Brown J et al. Developmental vitamin D3 deficiency alters the adult rat brain. Brain Res Bull 2005;**65**:141–8.
24. Myhr KM. Vitamin D treatment in multiple sclerosis. J Neurol Sci 2009;**286**:104–8.
25. Goldberg P, Fleming MC, Picard EH. Multiple sclerosis: decreased relapse rate through dietary supplementation with calcium, magnesium and vitamin D. Med Hypotheses 1986;**21**:193–200.
26. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;**296**:2832–8.
27. Munger KL, Zhang SM, O'relly E et al. Vitamin D intake and incidence of multiple sclerosis. Neurology 2004;**62**:60–5.
28. Salzer J, Svenningsson A, Sundstrøm P. Season of birth and multiple sclerosis in Sweden. Acta Neurol Scand 2010;**121**:20–3.
29. Kampman MT, Brustad M. Vitamin D: a candidate for the environmental effect in multiple sclerosis – observations from Norway. Neuroepidemiology 2008;**30**:140–146.
30. Correale J, Yssraelit MC, Gaitan MI. Immunomodulatory effects of vitamin D in multiple sclerosis. Brain 2009;**132**:1146–60.
31. Smolders J, Thewissen M, Peelen E et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PLoS ONE 2009;**4**:e6635.
32. Ramagopalan SV, Maugeri NJ, Handunnetthi L et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS Genet 2009;**5**:e1000369.
33. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol 2003;**134**:128–32.
34. Nashold FE, Spach KM, Spanier JA, Hayes CE. Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression. J Immunol 2009;**183**:3672–81.
35. Celius EG, Smestad C. Change in sex ratio, disease course and age at diagnosis in Oslo MS patients through seven decades. Acta Neurol Scand Suppl 2009;**189**:27–9.
36. Baas D, Prufer K, Ittel ME et al. Rat oligodendrocytes express the vitamin D(3) receptor and respond to 1,25-dihydroxyvitamin D(3). Glia 2000;**31**:59–68.
37. de Apreu DA, Eyles D, Feron F. Vitamin D, a neuroimmunomodulator: implications for neurodegenerative and autoimmune diseases. Psychoneuroendocrinology 2009;**34**(Suppl. 1):265–77.
38. Cass WA, Smith MP, Peters LE. Calcitriol protects against the dopamine- and serotonin-depleting effects of neurotoxic doses of methamphetamine. Ann NY Acad Sci 2006;**1074**:261–71.
39. Kalueff AV, Minasyan A, Keisala T, Kuuslathi M, Miettinen S, Tuohimaa P. Increased severity of chemically induced seizures in mice with partially deleted Vitamin D receptor gene. Neurosci Lett 2006;**394**:69–73.
40. Spector R, Johanson CE. Vitamin transport and homeostasis in mammalian brain: focus on Vitamins B and E. J Neurochem 2007;**103**:425–38.
41. Xiao F, Chen D, Lu Y et al. Proteomic analysis of cerebrospinal fluid from patients with idiopathic temporal lobe epilepsy. Brain Res 2009;**1255**:180–9.
42. Qin Z, Qin Y, Liu S. Alteration of DBP levels in CSF of patients with MS by proteomics analysis. Cell Mol Neurobiol 2009;**29**:203–10.
43. Ottervald J, Franzen B, Nilsson K et al. Multiple sclerosis: Identification and clinical evaluation of novel CSF biomarkers. J Proteomics 2010;**73**:1117–32.
44. Gascon-Barre M, Huet PM. Apparent [3H]1,25-dihydroxyvitamin D3 uptake by canine and rodent brain. Am J Physiol 1983;**244**:266–71.
45. Pardridge WM, Sakiyama R, Coty WA. Restricted transport of vitamin D and A derivatives through the rat blood-brain barrier. J Neurochem 1985;**44**:1138–41.
46. Balabanova S, Richter HP, Antoniadis G et al. 25-Hydroxyvitamin D, 24, 25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D in human cerebrospinal fluid. Klin Wochenschr 1984;**62**:1086–90.
47. Holmøy T, Moen SM, Gundersen TA et al. 25-hydroxyvitamin D in cerebrospinal fluid during relapse and remission of multiple sclerosis. Mult Scler 2009;**15**:1280–5.
48. Kremer R, Herscovitch P, Goltzman D. Detection of 25-hydroxyvitamin D in human cerebrospinal fluid. Clin Res 1980;**28**:692. (Abstract).
49. Torkildsen Ø, Knappskog PM, Nyland HI, Myhr KM. Vitamin D-dependent rickets as a possible risk factor for multiple sclerosis. Arch Neurol 2008;**65**:809–11.