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Vitamin D in the healthy and inflamed central nervous system: access and function

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ABSTRACT

High exposure to vitamin D may protect against development and progression of multiple sclerosis (MS), possibly through the immunomodulatory properties of its biologically active metabolite 1,25-dihydroxyvitamin D. So far, most studies on the possible mechanisms for vitamin D involvement in MS have focused on immune modulation outside the central nervous system (CNS). However, vitamin D may also interfere with the pathophysiology of MS within the CNS. In this review, the potential presence and functions of vitamin D in the inflamed and healthy CNS are explored. We discuss that vitamin D, vitamin D binding protein (DBP), the vitamin D receptor (VDR) and enzymes needed for metabolism (CYP27B1) are present in the CNS. Both VDR and CYP27B1 are expressed on a variety of cells, including neurons, glial cells, and invading lymphocytes. Additionally, vitamin D has been postulated to play a modulating role in several key-processes in MS pathophysiology, including inflammation, demyelination, axonal damage, and remyelination. We conclude that a local role of vitamin D in the inflamed CNS is likely and potentially relevant to MS. Future studies should further characterize the impact of vitamin D on the local disease process of MS in the CNS.

1. Introduction

A poor vitamin D status is an environmental risk factor for development of multiple sclerosis (MS) [1], but has also been associated with increased risk of relapses [2], advanced disability [3,4], and radiological measures of lesional injury and brain atrophy [5]. The most likely mechanism for vitamin D involvement in MS is believed to be modulation of immune responses by the biologically most active metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Expression of vitamin D receptors (VDR) on human immune cells were described more than 25 years ago [6], and was followed by the observations that $1,25(OH)_2D$ dampens T cell proliferation and prevents experimental autoimmune encephalomyelitis (EAE) [7,8]. It has recently been demonstrated *in vitro* that $1,25(OH)_2D$ regulates the expression of the MS associated DRB1*15 haplotype [9], and induces a regulatory phenotype of T cells [10,11]. Activated T cells express 1α-hydroxylase and are able to synthesize biologically active $1,25(OH)_2D$ from 25-hydroxyvitamin D (25(OH)D), the biologically inactive yet most stable and abundant vitamin D metabolite in the circulation [11]. Since T cell priming in MS and EAE takes place in the secondary lymphoid organs, vitamin D status has been proposed to interfere with the peripheral onset of these diseases [12]. Accordingly, the function of regulatory T cells in the blood from MS patients correlates with the serum levels of 25(OH)D [13,14].

Vitamin D may potentially not only interfere with immune responses in the periphery, but could also play a role in regulation of inflammation, neurodegeneration, and repair processes within the CNS [15]. The disease process in MS is secluded behind the blood brain barrier (BBB), which restricts the availability of vitamin D in the CNS. As the inflammation in MS, at least during the early phases of the disease, is likely initiated in the periphery, it may be difficult to separate intrathecal effects of vitamin D from the effects on the immune system in the periphery. The aim of this paper is to review whether the substrate, transport mechanisms and enzymatic machinery needed to obtain sufficient amounts of $1,25(OH)_2D$ are present in the CNS, and to discuss its potential implications in MS.

2. Vitamin D metabolites in the CNS

2.1. In healthy individuals

In the circulation, vitamin D is most abundant as its metabolite 25(OH)D. This metabolite is formed in the liver by hydroxylation of vitamin D, and has a long half-life. Approximately 100–1000 times

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lower concentrations of the biologically active metabolite 1,25(OH)$_2$D can also be found in the circulation. These levels are the result of hydroxylation of 25(OH)D in the kidney, and are driven by calcium homeostasis. The majority of the circulating vitamin D metabolites (99%) is tightly bound to vitamin D binding protein (DBP). DBP is predominantly synthesized in the liver, although substantial expression of DBP mRNA was also found in rat kidney, testis, abdominal fat, and yolk sac/placenta, but not in (non-inflamed) brain-tissue [16]. Interestingly, DBP is also found in CSF of healthy subjects [17–21].The gene encoding DBP is located on the same chromosome as albumin (chromosome 4), and DBP is also structurally closely related to albumin [22]. Therefore, the passage of DBP and subsequently DBP-bound vitamin D through the BBB is presumably as limited as that of albumin. Accordingly, early studies in rats have shown that the transport of 25(OH)D and 1,25(OH)$_2$D through the intact BBB is restricted [23].

An early study by Balabanova et al using a cyto-receptor assay reported that the concentrations of 25(OH)D and 1,25(OH)$_2$D in cerebrospinal fluid (CSF) of healthy humans were approximately 50% of those in serum, and also within the range of that quite commonly found in serum of healthy individuals [24]. The ratio of albumin concentrations in serum and CSF (serum:CSF ratio) generally exceeds 100:1, suggesting that particular transport mechanisms govern the vitamin D status within the CNS. We were not able to detect 25(OH)D in CSF with standard radioimmunoassay or liquid chromatography tandem mass spectroscopy, wich was surprising given the high concentrations reported by Balabanova *et al.* were well within the detection ranges of these assays [25]. In contrast to these findings, by using ultra performance liquid chromatography–mass spectroscopy, we found that the concentrations of 25-hydroxyvitamin D in CSF of patients with non-inflammatory neurological diseases were <1% of those in serum [25]. Although population specific differences cannot be fully excluded, our data make CSF concentrations of 25(OH)D in the range of those previously reported extremely unlikely [24]. The serum concentrations of 1,25(OH)$_2$D are generally 100–1000 times lower than 25(OH)D, and are therefore also likely to be less abundant than 25(OH)D in CSF [26]. Except for the Balabanova et al study, no data on 1,25(OH)$_2$D in the CNS or CSF of humans are currently available.

There are several mechanisms by which circulating 25(OH)D and 1,25(OH)$_2$D could theoretically access the CNS in healthy individuals (Fig. 1). The 'free hormone hypothesis' postulates that binding proteins in the circulation keep steroids in a biologically inactive state and regulate the circulating concentration of free hormones which can enter cells by diffusion [27]. According to this model, free concentrations of 25(OH)D and 1,25(OH)$_2$D in the serum could pass through the BBB and reach the CNS. Since only 0.40% of all vitamin D metabolites is unbound in the circulation, this would mean that only a very limited amount is available for entering the CNS [28]. Furthermore, this model seems unlikely as exclusive transport mechanism, since substantial amounts of DBP (presumably 25(OH)D-bound) are found in the CSF of healthy subjects [17–21]. Since most DBP in the circulation is synthesized by the liver, an active transport of vitamin D metabolites through the BBB has been postulated [29]. In several tissues, specific transport mechanisms have been described for the transportation of the vitamin D metabolite (vitD)–DBP-complex into target cells. In the kidneys, reabsorbtion of vitD–DBP-complex is dependent on the molecules Megalin and Cubulin at the luminal side of the proximal tubuli [30,31]. In mouse models, knock-out or inhibition of Megalin induced a severe vitamin D deficiency with a substantial loss of 25(OH)D via the urine [30]. Interestingly, rat cerebral microvessel endothelial cells of the choroid plexus display a diffuse cellular staining for Megalin [32]. Megalin-mediated [^{125}I]-RAP transport from the circulation to brain parenchyma has been described in mice [33]. Accordingly, Megalin-mediated transport of soluble amyloid-β-protein and apolipoprotein J into the CNS has been found in guinea pig choroid plexus cells [34]. Therefore, Megalin-dependent transport in the choroid plexus could be important for the crossing of

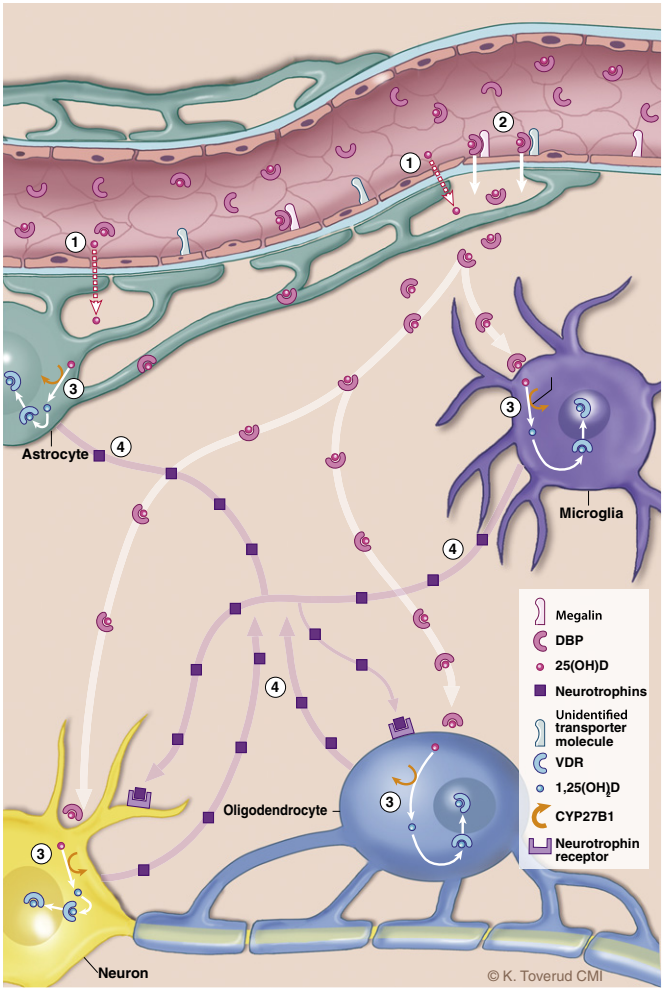

Fig. 1. Model of vitamin D transport and processing in the CNS in a healthy state. Vitamin D metabolites and DBP leave the circulation either via diffusion of free molecules [1], or via active transport by possibly Megalin or another transporter molecule [2]. In the CNS, 25(OH)D is processed by neurons and glia to 1,25(OH)$_2$D [3]. Neurons and glia all express the VDR, which ligates to auto- or paracrinely synthesized 1,25(OH)$_2$D. This results in a promoted neurite outgrowth, maturation and differentiation, and a promoted release of neurotrophins, as well as an enhanced expression of their receptors [4].

Vit D-DBP complex through the blood-CSF barrier. Interestingly, intense expression of VDR has been shown in the choroid plexus in early animal studies, suggesting that vitamin D transport into the CNS is a well regulated mechanism in healthy individuals [35,36].

2.2. In multiple sclerosis

Only a single study assessed the CSF levels of 25(OH)D in MS. We found that intrathecal 25(OH)D levels were comparable between RRMS patients and patients with other neurological diseases [25]. The CSF:serum ratio of 25(OH)D ([Q25(OH)D$_{CSF:serum}$]/[QAlbumin$_{CSF:serum}$]) was close to one in both patients and controls, and the CSF concentrations of 25(OH)D correlated positively with BBB integrity (QAlbumin) [25]. This finding indicates that a disrupted BBB could ease the transportation of DBP-bound vitamin D metabolites to the CNS (Fig. 2). Accordingly, several proteomic studies have shown elevated levels of DBP the CSF of MS patients [17,18], and CSF levels of DBP have also been suggested as a biomarker of BBB integrity in MS [37]. However, a focal (potentially inflammation-enhanced) expression of DBP by cells within the CNS cannot be ruled out [16,22]. Additionally, the data on DBP in the CSF of MS patients are not consistent. Thus, proteomoc screening data suggesting that an up-regulation of DBP predicts conversion from clinically isolated syndrome (CIS) to MS was not confirmed by enzyme-