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REVIEW

Vitamin D, Neurosteroids and Autism

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Summary

Vitamin D had been for a long time investigated for its effects on bone metabolism. Recently has been observed that the incidence of some neurodevelopmental disorders (including autism) increases hand in hand with vitamin D deficiency. Indeed, vitamin D was reported to modulate the biosynthesis of neurotransmitters and neurotrophic factors; moreover, its receptor was found in the central nervous system. Vitamin D deficiency was therefore assessed as a risk factor for autism, however the biological mechanism has not yet been revealed. In our review we focused on potential connections among vitamin D, steroids and autism. Potential mechanisms of vitamin D action are also discussed.

Key words

Vitamin D • Neurosteroids • Autism • ASD • Autoimmunity

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Introduction

The rapid increase of the prevalence of neuropsychiatric disorders suggests that less active indoor lifestyle and exposure to chemicals may impact the development of these diseases. Following chemicals or nutrients took attention to their association with neuropsychiatric disorders: smoke/tobacco, alcohol, air pollution, pesticides, endocrine-disrupting chemicals, heavy metals, micronutrients, fatty acid, and parental obesity as a proxy of accumulation of specific chemicals or nutritional status. Several chemical exposures may possibly be associated with an increased risk of neuropsychiatric disorders, whereas other traditional risk factors are less likely to be associated with the development of these diseases (Fujiwara *et al.* 2016). In our review we focus on vitamin D that is without any exaggeration contemporary phenomenon mentioned in connection with likely all areas of human health ranged from bone and glucose metabolism to mental health.

Vitamin D as a hormone

Vitamin D is an active steroid obtained by dietary uptake or synthesized in human skin from provitamin 7-dehydrocholesterol after the sunlight exposure. Further transformation, the first in the liver and then in the kidney yields hydroxyderivatives prehormone calcidiol (1(OH)D) and hormonally active calcitriol ($1,25\text{-}(OH)_2D$) and more than 35 structurally related less active hydroxycompounds. $1,25\text{-}(OH)_2D$ binds to the vitamin D receptors (VDR), thereby activating this receptor and regulating gene transcription. Concomitantly, non-genomic mechanisms of D-vitamin action that may involve L-type calcium channels have influence on brain function (Cui *et al.* 2015).

More than 85 % of circulating vitamin D metabolites are tightly bound to vitamin D-binding protein (VDBP) and only the fraction not bound to VDBP (free and albumin-bound vitamin D) is believed to be biologically active (Bhan *et al.* 2012). VDBP levels rise under certain conditions such as pregnancy (Bikle *et al.* 1984) and Alzheimer´s disease (Rinaldi *et al.* 2015) whereas decreased levels of VDBP were found for

example in hepatic diseases (Bikle *et al.* 1984).

Sometimes misunderstandings arise because the studies of ingested vitamin D do not distinguish among animal cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) that is plant origin and other forms of vitamin D. Level of plasma calcidiol is considered to be the optimal indicator of vitamin D status; however, biologically active form of vitamin D is calcitriol. A method for assessing vitamin D status has not yet been standardized.

Vitamin D and the brain

In addition to maintaining calcium/phosphate homeostasis, vitamin D promotes several health benefits including neuroprotective effects, especially by antioxidant activity, neuronal calcium regulation, neurotransmitter regulation, influence on several neurotrophic factors and immunomodulation. Vitamin D deficiency leads to disturbance of these processes and may be involved in the development of autism. It seems likely that vitamin D exerts the strongest effects on nervous system during the prenatal development and early infancy, but also relate to the adult subject´s mental state. The exact mechanism is waiting to be revealed, however, steroid hormones, vitamin D, their direct as well as epigenetic effects are presumably implicated in these processes.

The VDR and the enzyme 1α-hydroxylase are also expressed in other tissues, including neuronal and glial cells (Eyles *et al.* 2005). There is evidence that vitamin D has a neuroprotective effect on brain function.

Calcitriol 1,25-$(OH)_2$D inhibits the synthesis of inducible nitric oxide synthase (iNOS), which catalyzes nitric oxide, a free radical that can damage cells (Garcion *et al.* 2002). 1,25-$(OH)_2$D also stimulates γ-glutamyl transpeptidase activity, which is important in the synthesis of glutathione, an antioxidant and free radicals scavenger. In addition, vitamin D may act as a neurotrophic factor.

Already three decades ago it was shown (Naveilhan *et al.* 1996) that vitamin D stimulates brain cells to produce several growth factors, like nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and neurotrophin 3 (NT3). These neurotrophic and neuroprotective actions of vitamin D suggest that this vitamin can stimulate the protection and growth of neuronal cells. Therefore, vitamin D may slowdown the progression of neurodegenerative diseases.

In the brain, vitamin D has important roles in proliferation and differentiation, calcium signaling and neurotrophic and neuroprotective actions; it may also alter neurotransmission and synaptic plasticity. An increasing number of epidemiological studies indicate that vitamin D deficiency is associated with a wide range of neuropsychiatric disorders and neurodegenerative diseases (Groves *et al.* 2014, Kesby *et al.* 2011, Rimmelzwaan *et al.* 2016). There is robust evidence from rodent experiments indicating that transient developmental vitamin D deficiency is associated with changes in brain structure, neurochemistry, gene and protein expression and behavior. So the timing and duration of vitamin D deficiency in the 'critical window' of the development is decisive, however, it has not yet been properly defined (Cui *et al.* 2015). Based on animal models is speculated, that with regard to neurodevelopmental disorders the last trimester may be in humans the period most sensitive to low prenatal vitamin D (O'Loan *et al.* 2007). It is of interest that this critical window overlap a sensitive window for brain morphological alterations driven by gonadal steroids (McCarthy 2008).

Autistic spectrum disorder – ASD

Autism or autistic spectrum disorder (ASD) comprises wide range of neurodevelopmental disorders characterized by problems with social skills, repetitive behaviors, speech and nonverbal communication, as well as by unique abilities. According to *Diagnostic and Statistical Manual of Mental Disorders* version 5 (DSM-5), ASD includes autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger´s syndrome. In Czech Republic as well as in other countries, slightly different *International Statistical Classification of Diseases and Related Health Problems* version 10 (ICD-10) by the World Health Organization (WHO) is used for autism classification and diagnosis.

ASD has currently gained the characteristics of epidemic diseases. It was recently documented that the incidence of autism has grown by 700 % since the 1970s (Elsabbagh *et al.* 2012). Actual prevalence according to CDC (Centers for Disease Control and Prevention) is 1:68 and the cause of this increase is unknown.

ASD is a neurodevelopmental disorder, involving both genetic and environmental risk factors. Autism has been associated with more than 440 gene

variants, however, about 70 % of cases have a cause that has not been linked directly to genetics (Schaaf and Zoghbi 2011). The disease is characterized by primarily behavioral symptoms and includes impaired reciprocal social interactions, communication deficits, and propensity for repetitive behaviors. The diagnosis is very difficult given to behavioral nature of the symptoms and heterogeneity of neurodevelopmental disturbances that are characteristic for the disease.

Current diagnosis is based on questionnaires, which are usually completed by parents or guardians (Ostatnikova 2010, Ostatnikova 2015). To date there is no biological or laboratory marker proving or disproving autism. Even genetic testing shows gene variants that only affect autism risk and cannot account for autism appearance. The autism cannot be recognized even by any physical features. The average age of newly detected patients is 4.51 years, but experts believe that the first symptoms of the disease appear about 12^{th} month of age and the screening tests can be performed at age 16^{th} to 18^{th} months. Early and accurate diagnosis then leads to early intervention and better prognosis for the patient with autism.

Vitamin D and autism

Vitamin D plays crucial neuroprotective roles in the developing brain. Its deficiency has been reported in neuropsychiatric disorders as autism, depression, multiple sclerosis, Alzheimer´s disease, Parkinson's disease, attention deficit hyperactivity disease etc. It has an important role in cell proliferation and differentiation, immunomodulation, regulation of neurotransmission and steroidogenesis. The effects of vitamin D on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease were described in a handful of papers (Eyles *et al.* 2013). Animal studies have suggested that transient prenatal vitamin D deficiency is associated with altered brain development. The potential neurobiological mechanisms linking prenatal vitamin D deficiency and autism were reviewed recently (Ali *et al.* 2016).

Cannell (2008) was the first to propose that vitamin D reduced risk of autism. He based that assertion in part on higher prevalence of autism in regions with lower sunlight, whether due to latitude or clouds. A later paper reported higher autism rates in regions of the U.S. West Coast with higher precipitation rates (Grant 2016). Children with autism generally have lower vitamin D concentrations as has been already reviewed (Cannell and Grant 2013, Kocovska *et al.* 2012). A recent ecological study of autism prevalence among those aged 6-17 years found significant inverse correlations with respect to solar UVB doses (Grant and Cannell 2013). A study in Australia found that maternal 25 hydroxylated vitamin D (25(OH)D)) concentration <49 nmol/l at 18 weeks of pregnancy was associated with a significantly increased risk of the offspring being diagnosed with autism. In 2013, Cannell raised the question, "Will vitamin D treat the core symptoms of autism?" (Cannell 2013). Several papers have reported that the answer is yes and the treatment effects are more pronounced in younger children with autism (Feng *et al.* 2017, Saad *et al.* 2016).

High doses (5000 IU/day) of vitamin D given to the gravid mothers of autistic siblings really reduce the incidence of autism in the descendants to 5 % in comparison with the common 20 % (Jia *et al.* 2015, Saad *et al.* 2016, Stubbs *et al.* 2016). Autism symptoms and global functioning may improve after vitamin D supplementation. Saad *et al.* (2016) concluded that vitamin D is inexpensive, readily available and safe and that it may have beneficial effects in ASD subjects, especially when the final serum level is more than 40 ng/ml. However, the studies included small numbers of cases and were uncontrolled. The only double-blinded randomized clinical trial proving the efficacy of vitamin D_3 in the dose 300 IU vitamin D_3/kg/day in 109 ASD patients has been carried out (Saad *et al.* 2016). Supplementation of vitamin D was well tolerated and the autism symptoms of the ASD children improved significantly, following 4-month vitamin D_3 supplementation, but not in the placebo group. In contrary, children with the Williams syndrome, who may have greatly elevated calcitriol levels in early infancy, usually have phenotypes that are the opposite of autism. Children with vitamin D deficient rickets have several autistic markers that apparently disappear with high-dose vitamin D treatment.

Taken together, majority of studies have shown a negative correlation between the risk for autism and vitamin D levels; however, some studies do not confirm that relationship as reviewed and meta-analyzed by Wang *et al.* (2016). Discrepancy among the results might be due to the fact that studies reported total vitamin D levels only and did investigate neither VDR nor VDBP.

Studies also did not distinguish between cholecalciferol ($vitD_3$) and ergocalciferol ($vitD_2$) metabolites. It is well known that vitamin D_2 which is of

vegetable origin is 30-50 % less efficient than human/animal vitamin D_3. Ingestion of vitD_2 resulted in an increase in serum concentrations of vitD_2 metabolites, accompanied by a comparable decrease in concentrations of vitD_3 metabolites (Biancuzzo *et al.* 2013). Moreover, the level of vitamins D_2 was higher and level of vitamins D_3 was lower in patients with other diseases compared to reference ranges for healthy subjects (Mao *et al.* 2016). It indicates that the origin of vitamin D metabolites should be considered when investigating the effects of this vitamin.

The importance of vitamin D in autism is also supported by genetic studies. A recent paper reported that parental and child alleles of the vitamin D receptor were significantly correlated with risk of autism, as was, in children, an allele of *CYP2R1*. That gene encodes production of 25-hydroxylase, the enzyme that converts vitamin D to calcidiol 25(OH)D. A recent paper analyzed how vitamin D could affect risk of autism through its effects on tryptophan and serotonin production. The disruption of the serotonergic system is one of the most consistent observations in patients with autism. Serotonin is a neurotransmitter which promotes prosocial behavior and assessment of emotions. Its inadequate levels led to neuroanatomical disorders with cognitive deficits in animal models (Mazer *et al.* 1997).

Steroids and autism

The lifetime prevalence of ASD is about 1 %. Autism refers higher male incidence with gender ratio 2-4 males to 1 female. According to the "extreme male brain" theory of autism (Baron-Cohen 2002), fetal sex steroids are predicted to be involved in the development of cognition and autism. Elevated levels of selected steroids including progesterone, 17α-hydroxy-progesterone, androstenedione, testosterone and cortisol were observed in amniotic fluid of ASD children (Baron-Cohen *et al.* 2015). In agreement with the above theory, a couple of studies on postnatal steroids in autistic patients found significantly higher levels of androgens in saliva (Majewska *et al.* 2014) and blood samples (Geier and Geier 2007) of autistic children. In contrast, other studies found no relationship between postnatal testosterone concentrations and autistic traits in toddlers (Auyeung *et al.* 2012, Kung *et al.* 2016). Together, the later findings arose hypothesis that prenatal (but not postnatal) androgen exposure is associated with the development of autistic traits in young children (Auyeung *et al.* 2012).

Indeed, gender specific behavior and brain morphology is determined by gonadal steroids during the early development and surprisingly, it is neither testosterone nor other androgens which are responsible for brain masculinization in male fetuses. According to "Aromatization hypothesis", gonadal testosterone diffuses into the brain where is locally aromatized to estradiol and estradiol then initiates the process of masculinization (McCarthy 2008).

Theory of the significance of steroid hormones in autism supports knowledge of another disorder, concretely Smith-Lemli-Opitz syndrome (SLOS). SLOS is a malformation caused by a deficiency of the last step of cholesterol biosynthesis. The enzymatic deficiency manifests biochemically by increased levels of 7-dehydrocholesterol and reduced levels of cholesterol (Porter and Herman 2011). Individuals with SLOS have a high incidence of autism (71-86 % of children with SLOS had ASD) and cholesterol supplementation led to rapid behavioral changes including partial remission of autistic spectrum behaviors. However, cholesterol levels following supplementation did not correlate with presence or severity of autistic symptoms (Sikora *et al.* 2006). Thus, if ASD is predominant behavioral profile in SLOS and autistic symptoms are improved after cholesterol supplementation, it might be caused by a disruption of cholesterol biosynthesis. It is plausible that the rapid behavioral responses are mediated by neurosteroids derived from cholesterol rather than a cholesterol alone, which does not cross brain blood barrier (Jurevics and Morell 1995).

Other factors and autism

Different view on the disease suggests autism as a result of oxidative stress. Oxidative stress markers tend to increase whereas levels of the most important antioxidant – glutathione – is decreased in autism (Ghanizadeh *et al.* 2012). Vitamin D was shown to exert antioxidant effects.

Cannell and Grant (2013) reported that vitamin D plays a major role in the immune system. Evidence exists of neuroglial activation and neuroinflammation in the brain of patients with autism (Cannell and Grant 2013). Vitamin D is important in up-regulating production of antioxidants including glutathione, superoxide dismutase, and thioredoxin reductase (Alvarez *et al.* 2014). Thus, by genetically

up-regulating antioxidants, vitamin D could reduce neuroglial activation and neuroinflammation. Several authors have reported autoimmune conditions in autism including the presence of maternal antibodies to fetal brain tissue (Goines and Van de Water 2010, Martinez-Cerdeno *et al.* 2016, Mostafa and Al-Ayadhi 2013). They also found vitamin D blood levels were inversely and strongly associated with the absolute blood level of those antibodies ($R=-0.86$, $p<0.001$).

Vitamin D has a major role in inducing T regulatory cells which have an effect on controlling antibodies contributing to autoimmune conditions (Cantorna and Mahon 2004). Thus, the vitamin D induced T regulatory cells may have a role in reducing autoimmune conditions and protecting the fetus.

Future directions and open questions

Over the recent years, the prevalence of ASD has abnormally risen for unknown reasons. Specialists speak about epidemic character of the disease, since the incidence has increased to 1:100 in children under 8 years of age. The origin of the disease has not yet been identified; however, the correct and early diagnosis and then an expert assistance help to achieve functional status of the patient, in the best cases comparable with that of healthy individuals. Last but not least, the correct diagnosis and cooperation of patient's parents with specialists lead also to reduction of social isolation of the whole patient´s family.

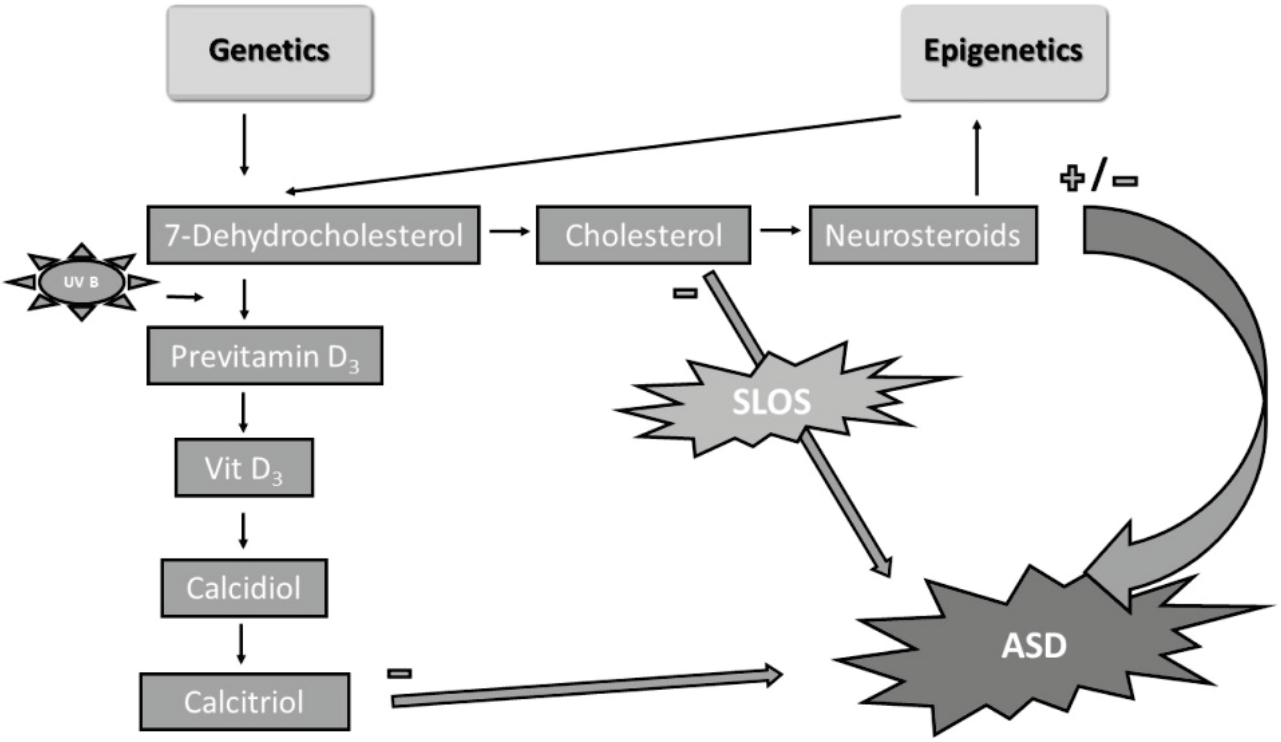

Fig. 1. Potential pathways of vitamin D and steroid hormones action related to ASD. ASD, autistic spectrum disorder; SLOS, Smith-Lemli Opitz syndrome; +/- indicates abnormal levels; - indicates deficiency.

Vitamin D deficiency is undoubtedly a risk factor for autism (Fig. 1), however, it turns out that the mere determination of vitamin D in blood is not sufficient marker to evaluate the vitamin D status. In cases where levels of VDBP alter, bioavailable vitamin D calculation may help to determine vitamin D status accurately (Kim *et al.* 2017).

Conflict of Interest

There is no conflict of interest.

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