VITAMIN D UpDates

VITAMIN D AND ISCHEMIC STROKE: Promise for prevention and improved outcomes

Leonardo Triggiani

Neurologist, Neurovascular Treatment Unit - Stroke Unit, Fabrizio Spaziani Hospital, Frosinone

Ischemic stroke produces irreversible damage in the brain and is one of the main causes of disability and mortality. In recent years, scientific research has progressively documented the role of vitamin D (VitD) in a wide range of physiological functions, beyond its classic role of regulating the homeostasis of calcium and phosphorus. In particular, it has been shown that VitD deficiency is associated with numerous chronic diseases, including cardiovascular, musculoskeletal, infective and autoimmune diseases as well as tumors. Low vitamin D levels are a common symptom in patients with cardiovascular pathologies such as ischemic stroke, myocardial infarction and hypertension; they are further linked to a greater risk of future cardio- and cerebrovascular events. Epidemiologic studies have demonstrated that VitD deficiency is a risk factor for stroke Patients who have suffered stroke show a high incidence of VitD deficiency, which may be attributed to reduced mobility and diminished exposure to sunlight, on one hand, and to an inadequate dietary regime, on the other. Reduced vitamin D levels can increase the risk of a future cerebrovascular event and contribute to functional deficits subsequent to a stroke. It is further necessary to note a seasonal variation in the incidence of ischemic stroke, with lower percentages during summer when exposure to sunlight allows for increased synthesis of active vitamin D metabolites.

This evidence may have important clinical implications, as high vitamin D levels can be useful for controlling cerebrovascular risk factors, such as high blood pressure, diabetes mellitus and metabolic syndrome; they may also produce antithrombotic and neuroprotective effects, such as a stimulation of neurotrophic factors, a reduction of oxidative stress, autoimmune response of the nervous system and regulation of the apoptosis, thereby reducing the risk of future stroke (Fig. 1). Currently available data indicate that vitamin D supplementation could represent a promising approach for preventing and treating stroke. Nonetheless, clinical trials that show a true association between vitamin D deficiency and stroke are still required; furthermore, trials are needed to establish whether vitamin D administration can reduce the incidence of stroke and the morbidity and mortality associated with it.

The aim of this review is to analyze currently available data on the correlation between VitD deficiency and cerebrovascular events; it further considers possible etiopathogenetic mechanisms and the use of vitamin D in stroke prevention and therapy.

CORRELATION BETWEEN HYPOVITAMINOSIS D AND CEREBROVASCULAR EVENTS

The Ludwigshafen Risk and Cardiovascular Health study (LURIC) [1], which involved over 3000 patients who underwent an angiography as well as a follow-up after an average of 8 years, showed that low vitamin D levels were a predictive factor for fatal stroke. In particular, after adjusting for possible confounding factors, the odds ratios remained significant for 25(OH)VitD at 0.67 (0.46, 0.97; p = 0.032), and for 1,25(OH)VitD at 0.72 (0.52, 0.99; p = 0.047). The authors suggest that vitamin D could have a protective effect against stroke, as the data indicate a negative association with hypertension, diabetes and atherosclerosis. Data from a population study have shown

Correspondence LEONARDO TRIGGIANI Itriggiani@gmail.com



FIGURE 1.

Role of vitamin D in the pathogenesis of cardiovascular events.

that elderly individuals with VitD deficiency are at an increased risk of future stroke, even after adjusting for age, gender, smoker status and functional capacity [2]. The risk turns out to be significantly lower for individuals in the highest and middle thirds of vitamin D intake compared to subjects in the lowest third (risk ratio 0.47, p = 0.011, and risk ratio 0.46, p = 0.024, respectively). In addition, vitamin D serum levels seemed to be predictive for stroke (risk ratio 0.41, p = 0.0053 in the highest third). For the authors, these findings indicate a real causal association between low vitamin D intake and future stroke; they consider supplementation as a promising approach for prevention.

The REGARDS study (Reasons for Geographic and Racial Differences in Stroke) [3], which was conducted on over 16,000 white and black patients, showed that those who lived in areas with less exposure to sunlight presented an increased stroke risk of roughly 56%. In addition, other researchers studied ca. 21,400 participants from the REGARDS study every 6 months over a 5-year period; they found that individuals with higher vitamin D levels in their diets had an 11% reduction in stroke risk and 24% lesser risk for cognitive decline. Such reductions held even after adjusting for factors for cardiovascular risk.

In any case, it is necessary to point out that the association between vitamin D, on one hand, and reduced risks for stroke and cognitive decline, on the other, could also be correlated to unmeasurable confounding factors, such as the fact that those with higher vitamin D levels potentially followed healthier diets.

Another study, based on data extrapolated from a medical database of 41,504 subjects [4], found that the prevalence of VitD deficiency (< 30 ng/mL) was 63.6%. VitD deficiency turned out to be closely associated (p < 0.0001) with an increased prevalence of hypertension, hyperlipidemia, diabetes and peripheral arterial disease. Levels of 25(OH)VitD were furthermore strongly linked to coronary disease, myocardial infarction, heart failure and stroke (p > 0.0001). Of particular interest were the increases in the prevalence of heart failure (90% relative and 9% absolute), myocardial infarction (81% relative and 2.6% absolute) and stroke (51% relative and 2%

absolute), as these individuals showed very low vitamin D levels with respect to controls (p trend < 0.0001 for all categories). In addition, very low vitamin D serum concentrations produced a higher composite relative risk for death, coronary disease, myocardial infarction, heart failure or cerebrovascular accidents (Hazard Ratio = 2.13, 95% Cl, 1.75, 2.58, p < 0.0001). A systematic review and meta-analysis has indicated that there was no significant reduction in mortality and cardiovascular risk associated with vitamin D levels (25(OH) VitD > 20 ng/mL) [5]. In particular, the review found no evidence for a significant link with outcomes for mortality, myocardial infarction and stroke, and no proof for the surrogate outcomes of hypertension, lipid fraction or glycaemia. Nonetheless, the authors acknowledged that the quality of the available evidence, in the best of cases, was low or moderate.

By contrast, another meta-analysis has provided evidence for an overall association between basal levels of 25(OH)VitD in the lowest categories (< 20 or 15 ng/mL) – compared with those in the highest (> 30 or 20 or 15 ng/mL) – and cardiovascular diseases (overall Hazard Ratio = 1.54, 95% CI, 1.22, 1.95) [6].

Evidence extrapolated from a review of the literature indicates that further clinical trials are necessary to verify an association between VitD deficiency and cerebral stroke.

POSSIBLE ETIOPATHOGENETIC MECHANISMS

Laboratory data suggest a potential causal association of VitD deficiency as a risk factor for stroke by means of a mechanism of systematic and vascular inflammation which determines – either directly or indirectly – atherogenesis (Table I). It has been shown that the activation of the nuclear receptor of vitamin D (VDR) can elicit *in vivo* antithrombotic effects, which suggests that the VDR system may play a physiological role in maintaining antithrombotic homeo-

TABLE I.

Association between vitamin D deficiency and cerebrovascular risk factors.

INDIRECT

- Hypertension
- Diabetes Mellitus
- Metabolic Syndrome
- Atherosclerosis

DIRECT

- Limitation of antithrombotic effects
- Platelet aggregation increase
- Tissue factor gene upregulation
- Antithrombin gene downregulation
- Thrombomodulin gene downregulation

Limitation of neuroprotective effects

- Neurotrophic factor biosynthesis:
 - Nerve growth factor (NGF)
 - Glial-derived neurotrophic factor (GDNF)
- Neurotransmitter biosynthesis
- Modes of brain detoxification:
 - Expression of inducible nitric oxide synthase (iNOS)
 - Intracellular levels of glutathione
 - Gamma-GT levels
- Modulation of neuron death:
 - Regulation of L-type voltage sensitive calcium channels (L-VSCCs) in hippocampal neurons

stasis. In an experimental study, the platelet aggregation induced by ADP showed a significant increase in VDR-knockout (VDR KO) mice with normal calcium levels. In addition, the genetic expression of antithrombin in the liver and that of thrombomodulin in the aorta, liver and kidney was down-regulated, while the expression of mRNA for the tissue factor in the liver and kidney was upregulated in VDR KO mice, independently of calcium plasma levels. Therefore, the vitamin D/VDR system increases the expression of antithrombotic factors and inhibits the expression of one of the thrombogenic factors, such as the tissue factor [7].

Another study, in which female rats were fed a diet with low levels of vitamin D (VDD) for 8 weeks, showed that the animals presented significantly greater cortical infarction volumes compared with the control group; they further had a more severe post-stroke behavioral deficit [8]. These findings were in part attributed to lower levels of Insulin Growth Factor-1 (IGF-1) in the cerebral tissue of the VDD rats, which normally plays a neuroprotective and bioregulatory role in ischemia; and in part to the involvement of an inflammatory response, with subsequent interleukin 6 (II-6) upregulation induced by ischemia after a VDD diet.

One of the hypothesized actions of vitamin D in the central nervous system, mediated through the influence of the active form of vitamin D, is that it modifies the production and release of neurotrophic factors, such as the nerve growth factor (NGF), essential for neuronal differentiation; vitamin D is further believed to be responsible for the increase in levels of the glial cell line-derived neurotrophic factor (GDNF) [9].

In this context, researchers using an experimental model of cortical infarction in rats reported a significant reduction of the infarcted area (which was obtained through the ligament of the middle cerebral artery) in animals which had received an intraperitoneal injection of 1 $\mu g/kg/day$ of 1,25(OH2)D for 8 consecutive days, resulting in a significant reduction of the volume and scope of infarction. Consequently, vitamin D pretreatment significantly increased the levels of cortical GDNF, generating the hypothesis that vitamin D reduces cerebral ischemia by means of GDNF [10].

Calcium homeostasis is essential for neuronal physiology, as an excess of calcium in neurons can contribute to neurotoxicity. Vitamin D has also been considered a

modulator of the opening of type-L calcium channels through non genomic effects by means of various kinases and enzymatic activity in the cerebral cortex [11]. Previous studies demonstrated that the glutamate receptor type N-methyl-D-aspartate (NMDAR) can promote neural survival [12]. In a previous model of ischemia damage in the brain of rats, intraperitoneal treatment with calcitriol for 6 consecutive days (with doses of 2 μ g/kg) produced a significantly reduced infarcted area and volume compared to controls (p < 0.01). This effect was correlated to a significant increase of the NMDA subunit and of the NR3A-MEK/ ERK-CREB pathway [13].

The integrity of the hematoencephalic barrier is of vital importance in reducing the neuronal damage following ischemic stroke. In an *in vitro* model of hypoxia utilizing bEnd. 3 cells, treatment with 1,25(OH2)D preserved the function of the hematoencephalic barrier (BEE) by means of activation of vitamin D receptors (VDRs) through NF-Kb, proving a protective effect, mediated by the VDRs, of vitamin D against the BEE dysregulation induced by the ischemia [14].

THE USE OF VITAMIN D IN STROKE PREVENTION AND THERAPY

Currently, very few trials are available that were specifically designed to study the effect of the administration of vitamin D for preventing stroke. A study which tested the possibility that vitamin D supplementation could improve some vascular markers (hypertension, cholesterol, B-type natriuretic peptide, heart rhythm disorder) in patients with previous ischemic stroke did not reveal statistically significant effects [15]. Nonetheless, the endothelial function, measured as flow mediated dilatation (FMD), showed significant improvement after 8 weeks of vitamin D supplementation compared with the placebo group. This finding turns out to be quite important, as endothelial dysfunction paves the way for atherosclerosis; it is also an independent risk factor for future cerebrovascular events.

In the Women's Health Initiative study, 36,282 menopausal women were divided into two groups: the first took 1000 mg of calcium and 400 IU of vitamin D a day, while the other took a placebo [16]. During the 7-year follow-up, 739 strokes occurred: the Hazard Ratio (HR) of the treated group with respect to the placebo was 0.95 (0.82, 1.10). With regard to the analysis of fatal cerebrovascular events (n = 114), the HR was 0.89 (0.62, 1.29) in the treated group compared to the placebo. The primary limitation of the study was the extremely low daily dose of 400 IU of vitamin D.

Recently, the findings of the ViDA trial have been published, a study conducted on 5,108 individuals who were given an initial dose of vitamin D3 of 200000 IU. followed - beginning a month later - by a monthly dose of 100000 IU or of placebo, for an average of 3.3 years (range 2.5-4.2 years) [17]. The primary outcome (cardiovascular diseases) was found in 303 participants (11.8%) of the group who took vitamin D, and in 293 (11.5%) of the placebo group, with an adjusted hazard ratio of 1.02 (CI 95%: 0.87, 1.20). The same results were observed in participants who had baseline VitD deficiency and for secondary outcomes. The authors conclude that monthly supplementation of high doses of vitamin D does not prevent cardiovascular diseases, but that further studies are necessary to ascertain the effects of daily or weekly vitamin D administration on cardiovascular risk.

With the aim of verifying the use of vitamin D supplementation in preventing cerebrovascular events, several large-scale clinical trials have recently begun [18]. The American study VITamin D and OmegA-3 TriaL (VITAL), which is currently underway, involves 25,871 patients of both sexes (men > 50 years of age and women > 55). It aims to investigate whether daily intake of 2000 IU of vitamin D or of omega-3 fatty acids reduces the risk of developing cancer, cardiovascular disease or stroke in patients with negative anamneses for these pathologies. The first results are expected sometime this year.

The Finnish Vitamin D Trial (FIND), another 5-year study, is being conducted on 2,500 participants (men > 59 years of age and women > 64) divided into three groups: 1) 1600 IU of vitamin D3/day; 2) 3200 IU/day; 3) placebo. The main outcomes of the study regard the prevention of cancer, cardiovascular diseases and diabetes. The results should be available in 2020.

CONCLUSIONS

Observational studies indicate that vitamin D might play a protective role against stroke. Nonetheless, only a few interventional studies are currently available, and each of these has methodological limitations. To the Aim of identifying the potential benefits of vitamin D supplementation on stroke incidence and outcomes, further studies are necessary; scientists will be able to extrapolate useful information when data from trials currently in progress are available. Available data seem to indicate that vitamin D can represent a safe and cost-effective preventive and therapeutic approach for patients with VitD deficiency (< 30 ng/mL) associated with other cerebrovascular risk factors or who have had ischemic stroke [19].

References

- Pilz S, Dobnig H, Fischer JE, et al. Low vitamin d levels predict stroke in patients referred to coronary angiography. Stroke 2008;39:2611-3.
- ² Marniemi J, Alanen E, Impivaara O, et al. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. Nutr Metab Cardiovasc Dis 2005;15:188-97.
- ³ Kent ST. The use of ground and satellite data to determine the relationship between long and short-term sunlight exposure with stroke incidence in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. International Stroke Conference 2012 (abstract 2591).
- ⁴ Anderson JL, May HT, Horne BD, et al. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am J Cardiol 2010;106: 963-8.
- ⁵ Elamin MB, Abu Elnour NO, Elamin KB, et al. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:1931-42.
- ⁶ Grandi NC, Breitling LP, Brenner H. Vitamin D and cardiovascular disease: systematic review and meta-analysis of prospective studies. Prev Med 2010;51:228-33.
- ⁷ Aihara K, Azuma H, Akaike M, et al. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. J Biol Chem 2004;279:35798-802.

- ⁸ Balden R, Selvamani A, Sohrabji F. Vitamin D deficiency exacerbates experimental stroke injury and dysregulates ischemia-induced inflammation in adult rats. Endocrinology 2012;153:2420-35.
- ⁹ Wrzosek M, Łukaszkiewicz J, Wrzosek M, et al. Vitamin D and the central nervous system. Pharmacol Rep 2013;65:271-8.
- ¹⁰ Wang Y, Chiang YH, Su TP, et al. Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. Neuropharmacology 2000;39:873-80.
- ¹¹ Brewer LD, Thibault V, Chen KC, et al. D Vitamin, Hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. J Neurosci 2001;21:98-108.
- ¹² Balázs R, Hack N, Jørgensen OS. Stimulation of the N-methyl-D-aspartate receptor has a trophic effect on differentiating cerebellar granule cells. Neurosci Lett 1988;87:80-6.
- ¹³ Fu J, Xue R, Gu J, et al. Neuroprotective effect of calcitriol on ischemic/reperfusion injury through the NR3A/CREB pathways in the rat hippocampus. Mol Med Rep 2013;8:1708-14.
- ¹⁴ Won S, Sayeed I, Peterson BL, et al. Vitamin D prevents hypoxia/reoxygenation-induced blood brain barrier disruption via vitamin D receptor-mediated/NF-kB signaling pathways. PLoS One 2015;10:e0122821.
- ¹⁵ Witham MD, Dove FJ, Sugden JA, et al. The effect of vitamin D replacement on markers of vascular health in stroke patients - A randomised controlled trial. Nutr Metab Cardiovasc Dis 2010;22:864-70.
- ¹⁶ Hsia J, Heiss G, Ren H, et al. Women's Health Initiative investigators. Calcium/vitamin D supplementation and cardiovascular events. Circulation 2007;115:846-54.
- ¹⁷ Scragg R, Stewart AW, Waayer D, et al. Effect of monthly high-dose vitamin D Supplementation on cardiovascular disease in the vitamin D assessment study. A randomized clinical trial. JAMA Cardiol 2017;2:608-16.
- ¹⁸ Kupferschmidt K. Uncertain verdict as vitamin D goes on trial. Science 2012;337:1476-8.
- ¹⁹ SPREAD Stroke Prevention and Educational Awareness Diffusion. *Ictus cerebrale: Linee guida italiane di prevenzione e trattamento.* VIII edizione. Stesura del 21 luglio 2016.