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Review

New aspects of vitamin C during prenatal development

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Summary. Vitamin deficiency leads to a cascade of reactions that ultimately result in the development of disease. Vitamin C is synthesized from glucose in the liver of most mammals, with the exception of humans, non-human primates and guinea pigs (*Cavia porcelus*). Namely, because of the evolutionary loss of the gene for the synthesis of L-gulonolactone oxidase (Gulo gene), endogenous synthesis of vitamin C is no longer possible, and these mammals must consume vitamin C to survive. Vitamin C is directly involved in collagen synthesis and severe deficiencies result in scurvy, a postnatal form of this vitamin deficiency. Although the consequences of postnatal vitamin C deficiency are relatively clear, there is still insufficient data concerning the effects of vitamin C deficiency during the prenatal period. Recent data indicate the importance of collagen in basal membrane integrity and its possible disturbance during vitamin C depletion. We have developed a novel guinea pig model of prenatal pial basal membrane disturbance during prenatal deprivation of Vitamin C. Results indicate that disturbance of collagen synthesis induces breaches in the pial basement membrane. As a consequence, the Bergman glia connection is lost, and neuron migration disturbances occur with developing dysplasia of cerebellar cortex, which can be found in Lissencephaly type II. The fact that neither humans nor guinea pigs can synthesize vitamin C creates an opportunity for further research into the impact of prenatal deprivation of vitamin C in developing neuron migration disorders.

Keywords: brain, guinea pig, neuron migration disorder, prenatal, vitamin C.

INTRODUCTION

The most striking example of exogenously induced systemic loss of homeostasis is that due to a lack of vitamins and trace elements. Every vitamin deficiency leads to a cascade of reactions that ultimately results in the development of disease.

Although Vitamin C (ascorbic acid) is synthesized from glucose in the liver of most mammals, this is not the case in humans, non-human primates and guinea pigs (*Cavia porcelus*). Namely, due to the evolutionary loss of the gene for the synthesis of L-gulonolactone oxidase (Gulo gene), the endogenous synthesis of vitamin C is no longer possible (Sato and Udenfriend 1978). Thus, these mammals must intake vitamin C from food in order to survive (Nishikimi and Yagi 1996; Lodish and Berk 2000). Many years before the discovery of vitamin C, two Norwegian researchers, Axel Holst and Theodor Frolich reported the first experimental scurvy model in guinea pigs. Examining beriberi in sheep, while using the guinea pig as an animal model, they realized that using the same food developed a completely different clinical picture. In fact they had developed the first experimental model of scurvy, a disease that had already been encountered in humans. It was known that a lack of an essential substance caused the disease, but its identity was not uncovered until Albert Szent-Gyorgyi isolated vitamin C in 1930, for which he was awarded the Nobel Prize (Oudemans-van Straaten et al. 2014).

Some o critical functions of vitamin C are its importance in the biosynthesis of collagen, L-carnitine, and dopamine conversion to norepinephrine. It also improves the function of the immune system, facilitates enteral absorption of inorganic non-heme iron, and participates in the synthesis

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of cortisol and catecholamines (Rebouche 1991). Vitamin C is a powerful antioxidant that is soluble in water. Under physiological conditions, it functions as a potent reducing agent that binds efficiently to free radicals and thus creates weakly reactive ascorbyl radicals. These reactions are crucial for aerobic cells (Tsukaguchi et al. 1999; Arrigoni and De Tullio 2002; Combs Jr and McClung 2017).

VITAMIN C AND COLLAGEN BIOSYNTHESIS

Collagen is a family of extracellular matrix proteins that, in addition to maintaining tissue structure, also play a role in tissue adhesion and remodeling. Collagen is the most abundant protein in the human body, comprising over 35% of the total protein in the human body (Nishikimi et al. 1994; Prockop and Kivirikko 1995; Mahmoodian and Peterkofsky 1999; Myllyharju and Kivirikko 2004; Veit et al. 2006). Collagen is found in large quantities in cartilage, tendons, ligaments, bones, intervertebral discs, blood vessels, cornea and dentin. Vitamin C and copper are necessary for the synthesis of collagen, which takes place in the ribosomes (granular cytoplasmic reticulum) and the Golgi apparatus of fibroblasts. One of the critical steps in its synthesis is the hydroxylation of two amino acids, proline and lysine, for which vitamin C is necessary (Kalluri 2003; Pescucci et al. 2003; Szpak 2011).

PRENATAL AND PERINATAL VITAMIN C DEFICIENCY

In adulthood, vitamin C deficiency will lead to a disorder of collagen synthesis with subsequent development of scurvy (Nishikimi and Yagi 1996; Lodish and Berk 2000). However, the consequences caused by the Vitamin C deficiency during prenatal development period are always the subject of discussion. Since the guinea pig, like humans, does not have the ability to endogenously synthesize its own vitamin C, the use of this animal as an experimental model offers enormous possibilities. It has been shown that the use of a non-scorbutic dose of vitamin C (100 mg/kg) in pregnant female guinea pigs leads to a significant decrease in both plasma and brain concentrations in the fetus compared to the mother. While in control animals, the concentration in the fetus is always higher than in the mother. This research indicates that when the intake of vitamin C is restricted, the fetus suffers the most, because the limited supply of the vitamin is first utilized by the mother (Schjoldager et al. 2013). The negative impact of vitamin C deficiency is primarily reflected in the regularity of the basement membrane built predominantly of type IV collagen. Electron microscopic analysis indicate a pronounced reduction of the intercellular matrix in the aortic endothelium in scurvy guinea pigs (Gore et al. 1965). Namely, vitamin C deficiency causes morphological changes in the endothelial cells and smooth muscle cells of blood vessels in guinea pigs. The basement membrane of blood vessels formed as a product of endothelial cell secretion is damaged as a consequence of vitamin C deficiency. In scurvy, blood vessels become fragile leading to frequent bleeding. Of course, because of its widespread distribution, the synthesis of collagen affects all structures in the body in which it is located. Thus, scurvy develops after day 21 of vitamin C deficiency in adult guinea pigs, resulting in decreased collagen synthesis and bone density (Kipp et al. 1996). Wegger and Palludan (1994) worked on pigs with a hereditary inability to produce vitamin C (OD pigs), who were not given vitamin C for 24 to 38 days during different gestation periods. The authors describe changes in the form of hematomas on the fetal and maternal sides of the placenta. The fetuses were free of morphological malformations; however, ossification was severely disturbed. Swelling of the costochondral joint, subperiosteal bleeding and separation of the epiphyseal cartilage from spongiosis on the bones, ribs and limbs were observed. At the same time, a reduced number of osteoblasts with irregular osteons were observed under the microscopic (Wegger and Palludan 1994).

NEW ASPECTS OF THE VITAMIN C DEPENDENT BRAIN DEVELOPMENT

Recent studies discovered that a lack of vitamin C during the early postnatal period in guinea pigs results in a decreased density of hippocampal neurons (Tveden-Nyborg et al. 2009). Namely, intake of insufficient concentrations of vitamin C in guinea pigs aged 6 or 7 days for a period of two months leads to behavioral disorders and a decrease in the number of neurons available for hippocampal formation (subunits of the dentate gyrus, cornu ammonis 1, 2 and 3). The authors state that the brain of a young individual is more susceptible to oxidative stress damage because of its immaturity (Tveden-Nyborg et al. 2009). Also, new data indicate the importance of collagen IV in the stability of the pial basement membrane. This is evident in coll IV knockout mice where fragile PBM break and leading to over-migration of the underlying neurons. In addition to knockout experimental models of collagen IV disturbance, we demonstrated a new aspect of the importance of vitamin C in brain development in guinea pigs. Using an evolutionary knockout animal model for the GULO gene, we investigated the effects of vitamin C deficiency on collagen synthesis during the prenatal period of guinea pigs (Capo et al. 2015). On the 50th day of gestation, fetal weights in the vitamin C deprived group were significantly reduced compared with the control group. Also, some fetuses from the vitamin C deprived group displayed talipes equinovarus of the forelegs and hind legs

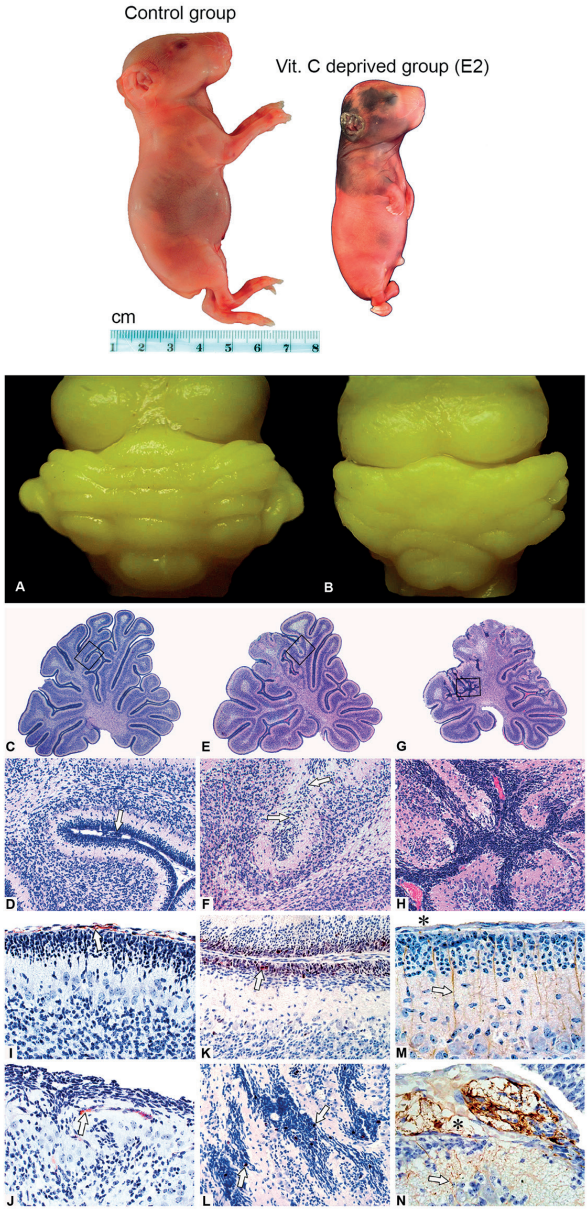

Fig. 1. Picture of a fetus from the control and vitamin C deprived group. Dorsal view of normal cerebellum in the control group (**A**) and absence of folia and sulci on the cerebellar surface in the vitamin C deprived group (**B**). Scoring of dysplasia: 1st stage - preserved structure of the cerebellar cortex without detectable lesions; 2nd stage - focal ectopic clusters of external granular layer (EGL) cells in subarachnoid space of the fissure from one folium and the pial membrane has minor damage (**C, D**); 3rd stage - focal to complete degradation of 1 or 2 fissures due to the fusion of opposing folia with loss of regular linear arrangement of Purkinje cells with a clear protrusion of the EGL and molecular layer into the subarachnoid space (**E, F**); 4th stage - the complete fusion of 3 or more fissures. The intensity of dysplastic changes in the cerebellum was most intense, in agreement with pathologic findings in lissencephaly type II (**G, H**). Staining collagen IV, Ki67 and GFAP in the control (**I, K, M**) and vitamin C deprived groups (**J, L, N**).

with visible muscular atrophy (Fig. 1 - fetus gross picture). The gross brain changes were most pronounced in the vermis with fused folia and a loss of borders between hemispheres and vermis, resulting in macroscopically visible flattening of the cerebellar surface (Fig. 1A, B). Microscopic disturbances include disorders in the composition of the pial membrane and the underlying pial membrane in the cerebellum. Different grades of dysplastic changes in the folia of the cerebellar cortex were assigned to different limits to mark the start and duration of vitamin C deprivation (Fig. 1C (2nd stage), E (3rd stage), G (4th stage)). Because of this damage to the above-mentioned structures, contact between the end legs of Bergman's glia and the basement membrane is lost, and neuronal migration is disturbed. Because of proliferation changes and the impossibility of migration through the radial glia, cells of the outer granulosa layer penetrate the damaged pial membrane and inhabit the intersulcus zones. Thus, sulcus spaces disappear, foliation is lost, and the brain's surface transforms from cobbled to flattened (Fig. 1D (2nd stage), F (3rd stage), H (4th stage)). Immunohistochemical findings show that in the control animal group, intact PBM immunopositive for collagen IV were detected (Fig. 1I, arrow), while in the deprived animal group, PBM was fragmented (Fig. 1J). Despite dysplastic changes in the cerebellar cortex, ectopic EGL cells retained anti–Ki-67 positivity (Fig. 1L). GFAP immunostaining found fragments of damaged Bergmann glial extensions (Fig. 1M, arrow) and gliosis of an ectopic mass of external granular layer cells in the subarachnoid space (Fig. 1N, arrow). All presented gross and microscopic changes can be found in clinical syndrome named Lissencephaly type II. This animal model provides new insight into the pathogenesis of Lissencephaly type II, and a new essential role for Vitamin C. Genetic causes are still cited as the cause of Lissencephaly type II, so researchers usually use a genetic model of this disease.

CONCLUDING REMARKS

Guinea pigs represent a unique animal model concerning prenatal vitamin C deprivation and associated significant disturbances in collagen synthesis. These findings indicate that vitamin C deficiency in the fetal guinea pig influences PBM rupture and sequential development of dysplastic changes in the cerebellar cortex. The fact that neither humans nor guinea pigs can synthesize vitamin C creates an opportunity for further research into the impact of prenatal deprivation of vitamin C in developing lissencephaly type II in humans.

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