LITERATURE REVIEW

The role of folate and one-carbon metabolism in brain development and hydrocephalus: a literature review

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Abstract

Folate metabolism has been known to influence the development of the nervous system, as found in the case of neural tube defects. Folates are a group of compounds involved in one-carbon metabolism, which is necessary for the formation of purine and thymidine nucleotides, as well as methionine and methyl donors. In addition to the well-documented role of folates within the pathogenesis of neural tube defects, current literature provides evidence that folate imbalances may play a significant role in the development and effects of hydrocephalus. This review considers the possibility that folate imbalances in hydrocephalic cerebrospinal fluid may be responsible for the neurological deficit seen in patients with this condition. Understanding the details of this potential imbalance may provide further insight into novel treatment options for hydrocephalus in the future.

Introduction

Harold Rekate defined hydrocephalus as 'an active distension of the ventricular system of the brain related to inadequate passage of cerebrospinal fluid (CSF) from its point of production within the ventricular system to its point of absorption into the systemic circulation'.[1] The condition is more common in infants, with the prevalence in Europe and North America being between 4.65 and 5.9 per 10,000 births.[2,3] Hydrocephalus can be congenital – for example, due to spina bifida, Arnold Chiari malformation or congenital aqueductal stenosis – or acquired after events such as intraventricular haemorrhage, meningitis or traumatic brain injury.[4] Hydrocephalus is classified into two types: communicating and non-communicating (obstructive),

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with each requiring a different approach to treatment.[5,6] In communicating hydrocephalus flow is not obstructed but CSF is not adequately reabsorbed in the subarachnoid space, whereas in non-communicating hydrocephalus flow of CSF from the ventricles is obstructed.[7] The condition may result in a raised intracranial pressure, or the intracranial pressure may remain relatively normal, as seen in normal-pressure hydrocephalus.[4] The clinical presentation of obstructive hydrocephalus is explained by the raised intracranial pressure, resulting in headache, vomiting and papilloedema. These features are less severe in young children as the cranial sutures have yet to fuse; the rising pressure is compensated by an increase in occipitofrontal circumference.[8] Normal-pressure hydrocephalus, on the other hand, presents later in life with the classic findings of Adam's triad: urinary incontinence, gait disturbance and dementia due to the effect of the enlarged ventricles putting pressure on the adjacent cortical tissue.[9] Diversion of CSF via a shunt is the primary treatment for both types of hydrocephalus; however, non-communicating hydrocephalus can also be treated via an endoscopic third ventriculostomy.[10,11]

The current literature provides evidence that a folate imbalance is present within hydrocephalic CSF, leading to complications in neurological development and function.[12,13] Folates are a group of compounds necessary for the synthesis of purine and thymidine nucleotides, methionine, and methyl donors via one-carbon metabolism.[14] Understanding the details of the imbalance could provide insight into alternative treatment options for hydrocephalus in the future. This paper reviews the current evidence regarding folate imbalance in the CSF and the potential effects on the pathophysiology of hydrocephalus.

One-carbon metabolism

The term 'one-carbon metabolism' describes a series of folate-dependent reactions that are involved in the formation of S-adenosylmethionine (SAM); conversion of serine to glycine; and synthesis of thymidylate, methionine and purines.[14]

Folate enters cells as 5-methyl-tetrahydrofolate (5-M-THF), and is demethylated to form tetrahydrofolate (THF) via the enzyme methionine synthase (metsyn).[14] During the first step, the 3 carbon of serine is transferred to THF by serine hydroxymethyl transferase (SHMT) to form 5,10-methylene-THF and glycine.[14,15] The 2 carbon of glycine is transferred to THF to form 5,10-methylene-THF, CO_2 and NH_4. 5,10-methlyene-THF is acted on by 5,10-methylene-tetrahydrofolate reductase (MTHFR) to produce 5-M-THF, which is crucial for methionine production. As well as this, 5,10-methylene-THF is required for thymidine synthesis as explained below. Therefore, its production by SHMT is a significant step in one-carbon metabolism.[14]

Purine nucleotide biosynthesis begins with 10-formyl-THF, which provides a one-carbon unit each to aminoimidazole-4-carboxamide and glycinamide ribonucleotide, becoming carbons 2 and 8 on the developing purine ring. In thymidine synthesis, 5,10-methylene-THF methylates deoxyuridylate monophosphate (dUMP), forming thymidylate monophosphate (TMP).[14] For every molecule of TMP required for DNA formation, a molecule of THF from 5-10-methylene-THF donates its one-carbon unit to dUMP and is oxidised to dihydrofolate (DHF) as a result.[16] Dihydrofolate reductase (DHFR) is responsible for converting DHF back to THF, and therefore maintaining TMP synthesis, as formation is sensitive to reduced levels of THF.[14,16]

Homocysteine (Hcy) is involved in two important pathways, methionine biosynthesis and transsulfuration to cystathionine. During transsulfuration, Hcy is condensed with serine to form cystathionine (C βS) which is then hydrolysed to cysteine via γ-cystathionase.[14] During methionine biosynthesis, 5,10-methylene-THF is reduced by MTHFR to form 5-M-THF, and the N-5 methyl group is used for transfer to Hcy, resulting in the generation of methionine.[14,15] In all cells, methionine is split between use for protein synthesis or for the formation of SAM.[17] SAM can be used as a methyl donor in a variety of reactions, where it is converted to S-adenosylhomocysteine (SAH), which is hydrolysed back to Hcy to begin the cycle again. When SAM levels drop, 5-M-THF formation is unrestricted while cystathionine formation is low, favouring the use of Hcy for methionine generation. Elevated SAM produces the opposite effect, favouring cystathionine production. Therefore, the SAM:SAH ratio is a very important determinant of one-carbon metabolism and the balance between the two pathways.[14] One-carbon metabolism is a very complex and essential pathway, and an image showing the reactions and compartments is provided in Figure 1.

Brain development

Human brain development begins at the third week of gestation and extends through to late adolescence.[18] This review will focus on the development of the ventricular system and the importance of folate in this process.

At the end of the third week of gestation, the embryo undergoes gastrulation and develops into a three-layered structure. The cells of the external layer are split into two types, the epidermal ectodermal layer and the neuroectodermal layer. The neuroectodermal layer contains neural stem cells that produce the cells of the brain and central nervous system (CNS), and are therefore known as neural progenitor cells.[18]

The neural tube forms between days 20 and 27 of gestation. At the end of gastrulation, the neural progenitor cells organise themselves along the

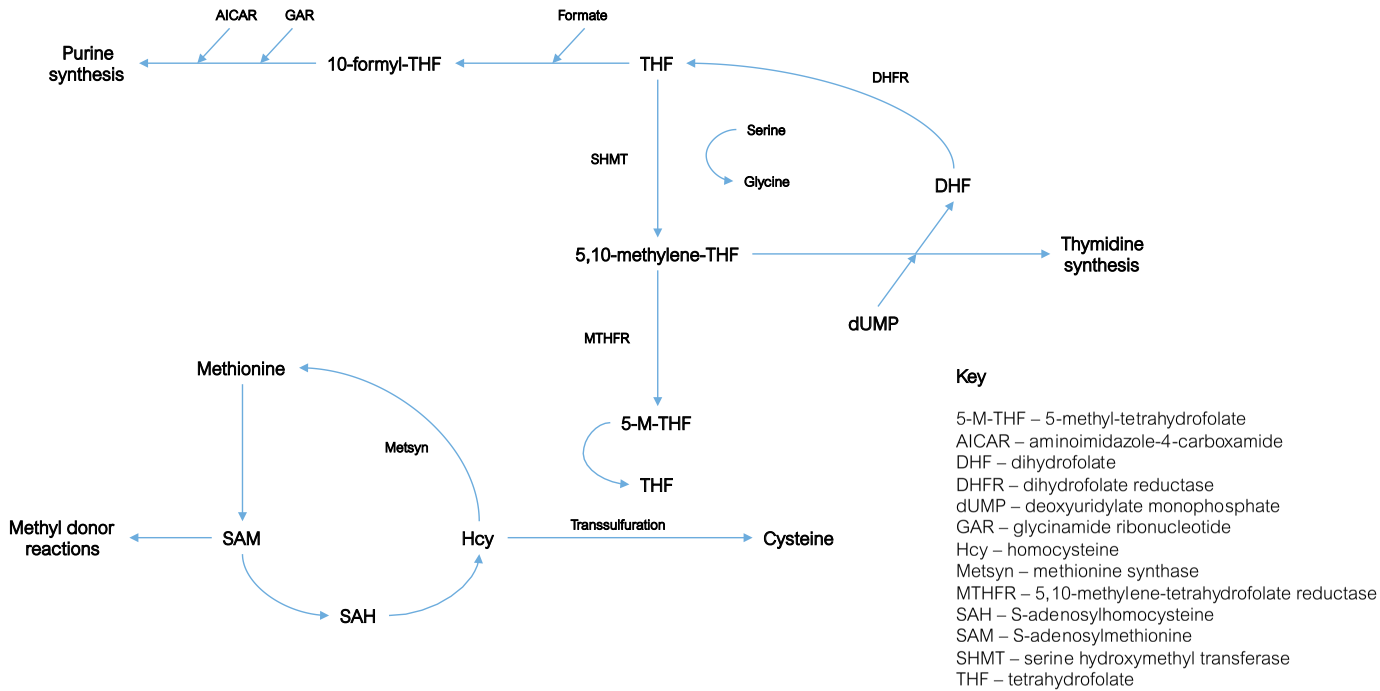

Figure 1: The folate pathway.

A schematic showing the reactions of folate metabolites involved with purine and thymidine synthesis and one-carbon metabolism.

Adapted from Lucock (2000), Bailey and Gregory (1999) and Hyde (2007).[14–16]

rostral–caudal midline, known as the neural plate.[18] At approximately day 21, a ridge forms on each side of the neural plate, and these rise, fold inward and fuse to form the neural tube.[19] Fusion of the neural tube begins at the centre, and proceeds in both rostral and caudal directions, with the rostral end (rostral neuropore) closing at approximately day 25, and the caudal end (caudal neuropore) at day 27. Neural progenitor cells line the tube, leaving a hollow cavity.[18]

Failure of neural tube closure falls into two categories: cranial dysraphism, which is due to the failure of cranial neural tube closure and includes anencephaly and encephalocele, and spinal dysraphism, which results from the failure of caudal neuropore closure, and includes spina bifida cystica and occulta.[20] As the embryo continues to develop, the rostral end of the tube forms the three primary brain vesicles: the prosencephalon, the mesencephalon and the rhombencephalon. As the shape of the neural tube changes, the shape of the hollow cavity within the tube also changes, going on to form the ventricular system.[18]

The role of folate in CNS development

In a study conducted by Kirke et al., it was shown that women carrying fetuses with neural tube defects (NTDs) had lower plasma levels of vitamin B12 and folate compared with women carrying healthy fetuses.[21] Study consensus indicates that folic acid supplementation during pregnancy results in an approximately fourfold reduction in the risk of NTDs.[22–24] The fortification of flour and other grain products with folic acid in the United States since 1998 has led to a 19% decrease in the prevalence of NTDs.[25] Other literature reports that an increased level of maternal homocysteine (a biomarker of impaired folate status/metabolism) is a risk factor for NTDs.[26,27] Genes playing a critical role in NTDs include those encoding MTHFR, metsyn reductase, C βS and folate receptors.[28] This suggests that genes involved with methionine and folate metabolism may be causative of NTDs.[20] Frosst et al. showed that 677C>T polymorphism results in a thermolabile version of MTHFR, leading to reduced enzymatic activity and elevated levels of homocysteine, which were lowered by folic acid supplementation.[29] Although this provides solid evidence that folate imbalances increase the risk of NTDs, the underlying mechanism by which supplementary folic acid reduces this risk remains unclear.[30]

Craciunescu et al. investigated the effects of a folate-deficient diet during late gestation on neural progenitors in the fetal mouse brain. They found that the number of progenitor cells in the developing brain septum, the caudate putamen and the neocortex was significantly lower in the folate-deficient embryos in comparison with the control. They also found that apoptosis was significantly greater in the dorsal regions of the developing

brain septum in the folate-deficient group compared with control. The change in diet between the groups of mice occurred during late gestation, showing that folate deficiency continues to have an effect on brain development long after neural tube closure.[31]

The ability of folate levels to affect the CNS is demonstrated in cases of cerebral folate deficiency, which is defined as any neurological syndrome associated with low levels of 5-M-THF in the CSF. Clinical features are present from the age of 4 months, with irritability and sleep disturbances, followed by cerebellar ataxia, spastic paraplegia, dyskinesia and epilepsy. Imaging has shown structural abnormalities in some children, such as atrophy of frontotemporal regions and periventricular demyelination.[32] Treatment with supplemental folate in the form of folinic acid is usually effective, and has been shown to improve the neurological status of some patients.[33] Cerebral folate deficiency demonstrates that folate levels in the CSF have an effect on brain function long after birth.

CSF secretion, composition and flow

CSF is secreted by the highly vascularised choroid plexuses within the lateral cerebral ventricles, which contain flattened cuboidal epithelia, an increased surface area, and increased levels of mitochondria and endoplasmic reticulum.[34–37] Secretion begins with the passive filtration of plasma from the choroidal capillaries to the interstitial compartment due to the hydrostatic pressure gradient between the blood and choroid interstitial fluid.[38–40] The second step consists of ion transport from the interstitial compartment to the apical surface.[38]

Secretion of CSF in adults is approximately 400–600 ml per day, but this varies between individuals and has been shown in rats to increase from birth to maturity.[38,41] However, this increase in secretion may not continue throughout the ageing process, as rat studies have found that the activity of choroidal ATPases, rate of Cl^- efflux and expression of aquaporin 1 decline significantly in older subjects, suggesting a reduction in overall secretory capability with age.[42,43] May et al. investigated the rate of CSF production in human subjects, and demonstrated an age-related decline in CSF production,[44] correlating with changes in the ageing choroid plexus.[42,43] Furthermore, it has been shown that patients with Alzheimer's disease have a reduction in CSF production.[45] Although that study had a small number of participants, it demonstrated that there is a possible role for CSF production rate in the aetiology of disease. While the study did not determine whether the change in CSF production is a cause or effect of the disease, it may be the case that the inability of newly formed CSF to reach other areas of the brain in hydrocephalus plays a role in neural function.

CSF produced in the lateral ventricles flows to the third ventricle via

the interventricular foramina, and through the cerebral aqueduct to the fourth ventricle.[38] CSF flow continues from the fourth ventricle through the medial foramen of Magendie and the lateral foramina of Luschka into the subarachnoid space, and then around the tentorium and upwards to the superior sagittal sinus, where most of it is absorbed. Some also flows downwards to the lumbar subarachnoid space, which is essential for fluid exchange and pressure–volume compensation.[46] In patients with Arnold Chiari I malformations, a cause of hydrocephalus, this downward CSF flow is disturbed and has been shown to result in bidirectional flow of CSF in the foramen magnum.[47] The flow disturbance seen may affect levels of CSF fluid exchange in the lumbar space and result in an imbalance of various compounds within hydrocephalic CSF.

Reabsorption of CSF into the peripheral lymphatic system is essential for the clearance of metabolites and waste products.[48] This occurs via arachnoid villi, which are endothelium-lined protrusions of arachnoid through the dura mater into the lumen of the venous sinuses.[38] In the case of communicating hydrocephalus, CSF cannot be reabsorbed adequately and this may result in the accumulation of metabolites and waste products, which may have an effect on cortical development and function.

Hydrocephalus and the role of CSF and folate

Although shunting relieves the pressure effects of abnormal CSF circulation, there are still long-term consequences of hydrocephalus. Many children born with obstructive hydrocephalus have reduced verbal intelligence quotient (IQ), and impairment in overall intelligence, learning and memory even after shunt treatment.[49] In the long term, it was found that up to 45% of treated patients suffered from depression requiring treatment, and that diagnosis of hydrocephalus at an earlier age leads to poorer long-term outcome.[50] However, it is unclear if these symptoms result from the disease process itself, or from the presence of a shunt. In the case of normal-pressure hydrocephalus, 59% of patients improve after shunting, with gait being the symptom most likely to improve, and cognitive impairment the least likely.[11] The continued presence of symptoms after shunting of hydrocephalus raises the question: if the CSF flow disturbance is resolved, why do neurological symptoms persist? It may be the case that the neurological deficit is the result of circulating factors within the hydrocephalic CSF.

Although CSF entry favours the ventral side of the brain, flow penetrates deeply into all brain areas, suggesting that CSF flow pathways may also serve a physiological role of parasynaptic volume transmission.[51] Gato et al. used explants from the mesencephalon of chicken embryos and observed the survival, differentiation and proliferation of the neuroepithelium.[52] They found that embryonic CSF prevented apoptosis and

increased proliferation, providing evidence that diffusible factors within the CSF have a significant role in neuroepithelial development. Further evidence was provided by Mashayekhi et al., who found that after cerebral aqueduct occlusion there was a significant decrease in cortical thickness, proliferation and cell number in the germinal matrix of hydrocephalic Texas (H-Tx) rat fetuses.[53] Furthermore, cells appeared to be cleared from the germinal matrix more rapidly, suggesting earlier migration. When cortical cells were removed from fetuses and placed in culture medium with no added growth factors, proliferation in the affected H-Tx cells was greater than that of the unaffected H-Tx or controls. This suggests that the affected cells were under inhibitory control in their in vivo environment, and once removed proliferation is then able to occur. In order to investigate this further, cortical cells were extracted from control Wistar rats and placed in culture for 24 hours, after which the culture medium was replaced with 10% CSF culture from the brains of each of the experimental groups. When cultured with control and unaffected H-Tx CSF, a threefold increase in the number of cells was observed after 96 hours. In the cultures with affected H-Tx CSF the cell number was similar to the starting cell number, suggesting that proliferation had been blocked, and that blockage of CSF flow results in the accumulation of an inhibitory signal, preventing cell proliferation. This may be the cause of the impaired brain development seen in hydrocephalus, rather than brain damage caused by raised intracranial pressure due to CSF accumulation.

Owen-Lynch et al. analysed the cell-cycle stage of samples taken from H-Tx rat fetuses at different stages of development, using Wistar rats as a control.[54] They found that cells taken from affected H-Tx rats were arrested in the S-phase of the cell cycle. This persisted for 24 hours after removal of the cells into culture, after which the cell-cycle distribution became similar to that of samples from the other groups. This supports the hypothesis that the in vivo conditions of the cells in affected H-Tx rats has a significant inhibitory influence, and shows that the cells from the affected H-Tx do not have an inherent proliferation defect and are otherwise normal. S-phase arrest can be induced using folate antagonists such as methotrexate, which are used clinically to treat malignancy.[55] These drugs have a high affinity for the DHFR enzyme and act as competitive inhibitors, disabling purine and thymidine synthesis. As well as this, methotrexate inhibits metsyn, reducing SAM levels, and thereby reducing the activity of DNA methyltransferases. It is possible that the S-phase cell-cycle arrest seen in hydrocephalic cortical cells may be related to imbalances of folate metabolism, leading to a lack of nucleotides.

The possibility of folate imbalance playing a role in hydrocephalus was investigated by Cains et al.[12] This study investigated the effects of folate derivative supplementation in vitro and in H-Tx rats in the mother, measuring levels of folate metabolites and enzymes involved in folate

metabolism. Dams were randomly selected and prescribed 2.25 mg/kg of various folate metabolites, including combinations of folinic acid and THF. Supplementation commenced 3 days prior to mating and was continued to the day of collection of the fetuses (day 20 of gestation). Cortical germinal epithelial cells of Sprague-Dawley rats were incubated with a variety of folate supplements in the presence of affected H-Tx rat CSF. Similar to the findings of earlier studies, affected H-Tx rat CSF reduced cell proliferation; however, thymidine supplementation caused a significant increase in cell proliferation that was not affected by the addition of affected H-Tx rat CSF. Furthermore, it was found that THF supplementation significantly reduced cell proliferation, which was also not affected by the addition of affected H-Tx CSF, demonstrating the possibility that the lack of proliferation seen in the cortex of hydrocephalic rats may be due to issues with folate metabolism. In vivo supplementation was compared to a saline-treated control which had a 33% incidence of hydrocephalus. It was found that supplementation of folic acid produced an incidence of 49%, folinic acid 30%, THF 25%, mix 1 (equal mixture of 2.25 mg/kg folinic acid and THF) 14%, and mix 2 (equal mixture of 4.5 mg/kg folinic acid and THF) produced an incidence of 24%. Folic acid produced a significant increase in incidence compared with the control, whereas mix 1 produced a significant decrease in incidence. This shows that folate imbalances may be the causative factor in the deficient structural development of the brain and ventricular system seen in hydrocephalus; however, the choice of supplementation is crucial.

Maternal supplementation was further investigated, to assess how it affected cortical development in the affected fetuses.[12] It was found that both affected and unaffected H-Tx rats had a significant reduction in cortical thickness compared with the control group. Mix 1 and mix 2 supplementation resulted in an increase in cortical thickness in unaffected H-Tx rats to control levels, and in H-Tx rats cortical thickness was also increased but not to the levels of treated unaffected H-Tx rats or controls. This same pattern was observed throughout the germinal epithelium, intermediate zone and cortical plate. Furthermore, it was found that both affected and unaffected T-Hx rats had lower levels of cortical cell proliferation compared with control groups, with affected H-Tx rats having the lowest levels. Both mix 1 and mix 2 significantly increased proliferation levels in the unaffected group, up to the levels of the control groups, and mix 2 resulted in a significant increase in proliferation in the affected H-Tx group; however, the levels did not reach those of the control group. Using nestin staining, it was found that maternal supplementation of both mix 1 and mix 2 increased differentiation across the entire cortex of affected H-Tx rats. This provides further evidence that the differences in cortical morphology and the lack of proliferation and differentiation seen in hydrocephalic H-Tx rats may be due to an imbalance in folate metabolites.

Increased expression of 10-formyl-THF dehydrogenase (FDH) has been linked to cell-cycle arrest and apoptosis,[56] and was therefore investigated further in a study conducted by Cains et al.[12] They found that affected T-Hx rats had reduced levels of FDH in the CSF compared with the unaffected H-Tx rats and Sprague-Dawley controls, and that levels were unaffected by folate supplementation in affected H-Tx rats.[12] They also investigated distribution of metsyn expression, and found that there appeared to be lower levels of metsyn expressed in the cortex of affected H-Tx rats compared with other groups, and there was significant staining in the choroid plexus in all groups.[12] FDH acts to stabilise and transport THF within the CSF, and although increased levels have been shown to induce cell-cycle arrest, the low levels seen in affected H-Tx rats appear to have a similar effect.[56] Low levels of FDH would reduce transport of THF, and increase the levels of free THF, which may result in the reduction of cell proliferation seen in THF supplementation that was mentioned earlier.

The role of folate metabolism in normal-pressure hydrocephalus has also been investigated. Woodruff et al. analysed the CSF of patients with normal-pressure hydrocephalus for levels of 5-M-THF, B6, B12, Hcy, C βS and FDH, and compared the results with those of controls.[13] They found that levels of 5-M-THF and C βS were reduced in the experimental group, whereas levels of FDH and Hcy were elevated. Reduced levels of 5-M-THF and increased homocysteine may result in hyperhomocysteinaemia, which has been linked to dementia, and may provide an insight into the cause of cognitive impairment seen in patients with normal-pressure hydrocephalus.[9,57] Raised levels of FDH have been shown to result in cell-cycle arrest and apoptosis, which may explain the neurological decline seen in patients.[56]

Conclusion

CSF clearly has a role in CNS development and function. The CSF flow and absorption pathway is important in the maintenance of CSF composition, and disturbances in this due to hydrocephalus can lead to a lack of fluid exchange and the build-up of inhibitory signals. Therefore, defects in the folate pathway could explain the neurological deficit seen in many patients after shunt treatment, both in childhood and in later life. Supplementation with certain folate enzymes or metabolites can either increase or decrease the incidence of hydrocephalus, demonstrating that folates play a significant role in this condition. Future research needs to enhance our understanding of the effects folate metabolites exert upon the neurodevelopmental process, and could lead to alternative treatments with greater efficacy than those available today.

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