

THE EFFECTS OF FOLIC ACID DEFICIENCY ON HIPPOCAMPAL MORPHOLOGY IN A MOUSE MODEL OF VASCULAR COGNITIVE IMPAIRMENT

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Abstract

Vascular Cognitive Impairment (VCI) is the most prevalent form of dementia after Alzheimer's Disease. A deficiency in folic acid, a B-vitamin, is a modifiable risk factor for neurodegeneration. The aim of the present study was to assess the impact of folic acid deficiency in combination with VCI on hippocampal morphology using a mouse model. Adult male mice were split into four experimental groups. Mice were placed on a control diet (CD) or folic acid deficient diet (FADD). Chronic hypoperfusion, which models VCI in mice, was induced by implanting microcoils around the common carotid arteries. The control mice had the microcoils placed beside the arteries. Hippocampal morphological analysis included measuring the thickness of the granular cell layer of the dentate gyrus. We hypothesize that a FADD in combination with chronic hypoperfusion should result in reduced thickness of the granular cell layer within the dentate gyrus of the hippocampus. Our results demonstrate that the thickness of the granular cell layer was increased in FADD microcoil mice. This data suggests that there may be an additional factor coming into play such as compensation by neural stem cells within the dentate gyrus. These results require further investigation.

La Déficience Cognitive Vasculaire (DCV) est la forme de démence la plus répandue après la maladie d'Alzheimer. Une carence en acide folique, une vitamine B, est un facteur de risque modifiable de la neurodégénérescence. Le but de cette étude était d'évaluer l'impact de la carence en acide folique en combinaison avec la DCV sur la morphologie de l'hippocampe en utilisant un modèle de souris. Des souris mâles adultes ont été réparties en quatre groupes expérimentaux. Les souris ont été soumises à un régime témoin (RT) ou à un régime déficient en acide folique (RDAF). L'hypoperfusion chronique, qui modélise la DCV chez la souris, a été induit par l'implantation de microbobines autour des artères carotides communes. Chez les souris régulations, microbobines ont été placées à côté des artères. L'analyse morphologique de l'hippocampe comprenait la mesure de l'épaisseur de la couche de cellules granulaires du gyrus denté. Nous émettons l'hypothèse qu'un RDAF en combinaison avec une hypoperfusion chronique devrait résulter d'une réduction de l'épaisseur de la couche de cellules granulaires dans le gyrus denté de l'hippocampe. Nos résultats démontrent que l'épaisseur de la couche de cellules granulaires a augmenté dans les souris à RDAF avec microbobines. Ces données suggèrent qu'il pourrait y avoir un facteur supplémentaire qui agit, comme la compensation par les cellules souches neurales dans le gyrus denté. Ces résultats nécessitent une enquête plus approfondie.

Keywords

Vascular Cognitive Impairment; Lesions; Hippocampus; Chronic Hypoperfusion; Folic Acid Deficiency



Introduction

Vascular cognitive impairment (VCI) is a type of dementia presumed to be caused by cerebrovascular disease (Iadecola, 2013; O'Brien et al., 2003). VCI is the second leading cause of dementia after Alzheimer's disease, making up 25-30% of dementia cases (O'Brien et al., 2003). The common symptoms of VCI include attentional impairments, slowed processing, executive dysfunction and depression, with symptoms varying from patient to patient (O'Brien et al., 2003). VCI is thought to worsen over time and includes individuals who have cognitive impairment related to stroke, multiple cortical infarcts, silent infarcts, and/or small vessel disease with white matter (WM) lesions (O'Brien et al., 2003; Shibata, Ohtani, Ihara, & Tomimoto, 2004). WM consists of myelinated axons and in the elderly cerebrovascular white matter lesions are correlated with cognitive impairment and gait disorders. (O'Brien et al., 2003; Shibata et al., 2004) It has been reported that cerebrovascular WM lesions also contributes to subcortical vascular dementia, which is a subtype of VCI (Shibata et al., 2004). Due to the complexity of the disease, there are limited treatment options in terms of cognition for VCI (Iadecola, 2013; O'Brien et al., 2003).

Alterations in the vasculature of the brain results in changes, such as hippocampal atrophy, the degeneration of the hippocampus, which is a component of the VCI subtype, subcortical vascular dementia (van de Pol, Gertz, Scheltens, & Wolf, 2011). The hippocampus plays an important role in learning and memory, and lesions to this area have a significant effect on memory (Squire, 1992; Wen et al., 2007). Hippocampal volume loss due to subcortical vascular impairment has been reported to a similar degree that is observed in Alzheimer's Disease (Wen et al., 2007).

Deficiencies in folic acid, the B-vitamin, have been reported to contribute to hippocampal atrophy in animal studies (Chen et al., 2001; Jadavji et al., 2012). Folic acid has been shown to have a significant effect on the supply of

substrates for genome maintenance proteins, and especially important to control gene expression and chromatin structure (Fenech, 2010). A folic acid deficient diet has been proven to damage nuclear and mitochondrial DNA, as it can disable the regenerative potential for normal tissue. This was shown in *in vitro* studies of hippocampal cell structures which found that cells have an increased probability of cell death, and hippocampal neurodegeneration when they are folic acid deficient (Kruman et al., 2002; Kruman, Mouton, Emokpae, Cutler, & Mattson, 2005). Increased levels of apoptosis and atrophy have also been reported in mice with genetic deficiencies in folic acid metabolism (Chen et al., 2001; Jadavji et al., 2012). It was suggested that the cause of this may be due to an increased level of oxidative stress during folic acid deficiency (Fenech, 2010).

Cerebrovascular WM lesions also contribute to subcortical vascular dementia, and to study the effects of these lesions an accurate animal model is required. Although the most ideal model would have been non-human primates since they are most similar to humans, mouse models are the most accurate and accessible model available for the early stage of research. Mouse models can be used to study human neurological diseases because of the similarity in brain structures and the ease of manipulation (Ellenbroek & Youn, 2016; Harper, 2010). In this model designed by Shibata et al., (2004) microcoils were used to induce bilateral common artery stenosis, resulting in cerebral hypoperfusion and WM lesions (Shibata et al., 2004). The microcoil implantation causes a progressive reduction in blood flow and the negative effects (e.g. weight loss) experienced by animals is minimal. More specifically, implantation of microcoils around the common carotid artery result in reduced blood flow to the brain, causing a progressive neurodegeneration (Shibata et al., 2007, 2004). This model has also been reported to result in hippocampal atrophy (Nishio et al., 2010), however, the impact of folic acid with this model remains unknown. Therefore, the aim of this study was to assess the impact of dietary folic

acid deficiency and reduced blood flow to the brain on hippocampal morphology. We hypothesized that microcoil implantation in combination with reduced levels of dietary folic acid would disrupt homeostasis and increase cell death of neurons within the dentate gyrus. This would then lead to a reduced thickness of the granular cell layer of the hippocampus.

Materials and Methods

Animal Experimentation

All experiments were in accordance to the Canadian Council on Animal Care guidelines. The male mice were four weeks of age when they were placed on either a control diet (CD) containing a 2 mg folic acid/kg diet or a folic acid deficient diet (FADD) which contained 0.3 mg folic acid/kg. The mice were maintained on the diets for six weeks before surgery. The animals per group ranged from 3 to 7. The mice were maintained in a controlled environment with a temperature of 22°C, 55% humidity and 12-hour light/dark cycle. At the completion of the experiment, brain tissue was collected, sectioned, and stained, after which hippocampal morphology was assessed using cresyl violet histological staining, details are described below. No animals became sick or died before the end of this experiment.

Chronic hypoperfusion via bilateral common carotid artery stenosis

While maintaining an oxygen temperature of 37.0°C, and a nitrous oxide to oxygen mixture of 70:30 the animals were anaesthetized with isoflurane (1.5%). The process was done using an automated heat blanket that maintained the temperature level. The two carotid arteries of the animals were made visible through a midline incision, while the animals were laid horizontally with the face and torso facing up. The microcoil procedure was completed by wrapping microcoils (180 Qm in inner diameter) around both carotid arteries. For the control (Sham) surgery mice had the microcoils placed beside

the carotid arteries (Jadavji et al., 2015). All animals were given 6 mg/mL of acetaminophen in the drinking water one day before, and up to three days after the surgery to relieve pain.

Hippocampus morphology

Once the experiments were completed, the mice were placed under anesthesia with isoflurane. Brain tissue was fixed in 4% paraformaldehyde after which the right hemisphere was sectioned at 5 Qm thick. Brain sections as stained using cresyl violet in order to assess hippocampal morphology and the thickness of the granular cell layer within the dentate gyrus. For each mouse, three measures were taken of the granular layer of the dentate gyrus region were taken using ImageJ software (NIH). The measurements were taken from three hippocampal sections and then averaged for each mouse

Statistics

Data analysis was performed by two individuals blinded to experimental groups. Statistical analysis was performed using Graph Pad Prism 6.0. A one-way analysis of variance (ANOVA) was used to compare the means of the four groups. All data are presented as mean \pm standard error of the mean (SEM). Statistical tests were performed using a significance level of 0.05.

Results

The experimental results showed the mice that underwent just the microcoil surgery procedure had a trend of increased thickness of the granular layer of the dentate gyrus (**Figure 1**, $F(1,16)=3.5$, $p=0.08$). There was no difference in the thickness of the granular cell layer between diet treatments ($F(1,16)=0.23$, $p=0.64$).



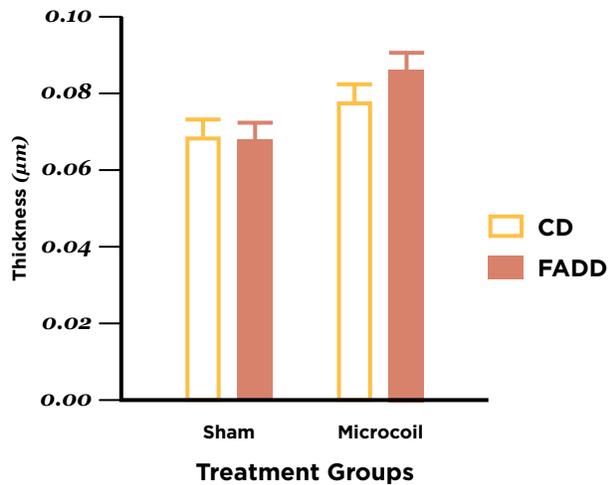


Figure 1: Impact of folic acid diet deficiency (FADD) on hippocampal morphology in a mouse model of VCI- *The thickness of the dentate gyrus within the hippocampus of mice placed on control diet (CD) and FADD with or without microcoil implantation.*

Discussion

This study evaluated hippocampal morphology in a mouse model with VCI with dietary folic acid deficiency. We observed a trend for increased granular layer thickness in the FADD microcoil mice when compared to the control animals, however it was not statistically significant.

The results of our study do not support our initial hypothesis which stated that chronic hypoperfusion in combination with reduced levels of dietary folic acid would disrupt homeostasis and increase cell death of neurons within the dentate gyrus, making the granular cell layer thinner. It is possible that increased levels of homocysteine and the chronic hypoperfusion procedure may not have impaired neurogenesis. (Ming & Song, 2011) A possible explanation for these non-significant results is the small sample size. A higher confidence interval such as 80% or 90%, was not achieved potentially due to the low sample size, resulting in too broad of a range to predict. Therefore, increasing the number of animals per group would perhaps allow for a more informative result.

It was also observed that the mice which underwent FADD and microcoil treatment did show a non-significant increase in thickness of the granular cell layer with the dentate gyrus after the microcoil implantation. Another possible explanation for an increase of thickness after the microcoil treatment, is that stem cells and neurogenesis within the hippocampus might have played a part in restoring damaged cell tissue (especially in the early stages), and therefore could have played a role in rescuing compromised cells (GRAMSBERGEN, 2007).

Conclusion

Our study suggests that a change in hippocampal morphology could potentially be affected by VCI, and the trends presented show that the topic merits further investigation. More research is required to investigate health of cells within the dentate gyrus and the rest of the hippocampus.

Abbreviations

Abbreviation	Full Form
VCI	Vascular Cognitive Impairment
CD	Control Diet
FADD	Folic Acid Deficient Diet
Sham	Control Surgery
WM	White Matter

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