Evidence of upregulation of the cholinergic anti-inflammatory pathway in late-life depression

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Abstract

Background: Decreased cholinergic tone associated with increased proinflammatory cytokines has been observed in several human diseases associated with low-grade inflammation. We examined if this attenuated cholinergic anti-inflammatory pathway (CAP) mechanism contributed to increased neuroinflammation observed in depression.

Methods: We measured cerebrospinal fluid (CSF) cholinergic markers (AChE and BChE activities) in 28 individuals with longstanding late-life major depression (LLMD) and 19 controls and their relationship to central and peripheral levels of pro-inflammatory cytokines (IL-6 and IL-8). Additionally, we examined if these cholinergic indices were related to CSF markers of microglial activation and neuroinflammation (sTREM2 and complement C3).

Results: Compared with controls, LLMD patients had a significant reduction in CSF BChE levels. Lower CSF BChE and AChE activities were associated with lower CSF markers of microglial and neuroinflammation (sTREM2 and C3). In addition, in LLMD patients we found an inverse relationship between peripheral marker of inflammation (plasma IL-6) and CSF BChE and AChE levels.

Conclusions: Our results suggest an upregulation of the CAP mechanism in LLMD with an elevation in peripheral markers of inflammation and concomitant reduction in markers of glial activation associated with a higher cholinergic tone. Future studies should confirm these findings in a larger sample including individuals with acute and more severe depressive episodes and across all ages.

Keywords: Acetylcholinesterase; Butyrylcholinesterase; C3; Cerebrospinal fluid; Cholinergic anti-inflammatory pathway; Late-life major depression.

Introduction

Numerous reports and meta-analyses have documented increases of various peripheral proinflammatory cytokines, such as interleukin 6 (IL-6) and interleukin 8 (IL-8) in depression, including late-life major depression (LLMD). These abnormalities are particularly prominent in drug-naïve individuals during their first episode of depression, in those with severe depression, suicidal ideation or a history of child abuse, and in patients with treatment-resistant depression. There is extensive evidence that treatment of medical conditions with cytokines, such as interferon-γ (IFN-γ), may result in the development of depression. Finally, diverse disorders associated with inflammation have also been reported to have a high incidence of depression. Therefore, it has been hypothesized that increases in peripheral inflammation may be etiologically linked to depression.

Conversely, several observations challenge the reliability of using peripheral levels of proinflammatory cytokines alone for the identification of individuals with inflammatory-type depression. For example, elevated plasma cytokines levels are neither necessary nor sufficient for the emergence of depressive symptoms, and peripheral cytokines do not generally correlate with corresponding cerebrospinal fluid (CSF) levels. Some studies have reported reductions in proinflammatory cytokines in depression rather than elevations, including two different studies in LLMD that found a significant reduction in plasma and CSF IL-6. Likewise, a study in schizophrenia, another disorder which may also be accompanied by an increased in peripheral inflammatory markers, reported that an increase in plasma IL-6, was not associated with neuroinflammation, as determined by the in vivo TSPO positron emission tomography (PET) signal.

In summary, it is necessary to find other biomarkers to use in conjunction with circulating proinflammatory cytokine levels to identify individuals with an inflammatory depression subtype more reliably. We propose that the analysis of central and peripheral indices of cholinergic activity may address this need. Extensive data from preclinical and human studies have demonstrated an important role of the central and peripheral cholinergic system in the regulation of the immune response. In brief, an enhancement of central cholinergic activity via administration of M_1 muscarinic acetylcholine (ACh) receptor (mAChR) agonists, or acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors, which cause an increase in brain ACh and cholinergic activity, has been demonstrated to result in a reduction in pro-inflammatory cytokines release and better clinical outcomes after acute exposure to endotoxin, sepsis and other inflammatory conditions. These anti-inflammatory effects have been shown to be mediated by increases in vagal efferent activity and ACh-mediated stimulation of α-7-nicotinic receptors (α7 nAChR) on macrophages, and other inflammatory cells which suppress the activation of the nuclear factor kappa B (NF-kB) signal transcription pathway and inhibit the production of proinflammatory cytokines. Importantly, reductions in peripheral BChE activity, associated with enhanced cholinergic activity, have been described as the earliest manifestation of acute systemic inflammation and have been associated with decreased release of pro-inflammatory cytokines as well as better survival. It has been suggested that reduction in peripheral BChE may reflect a

compensatory upregulation of the cholinergic anti-inflammatory pathway in response to increased systemic inflammation.

Thus, it is possible that the determination of central and peripheral cholinergic indices, in conjunction with corresponding plasma proinflammatory cytokine levels, may help to identify more reliably those individuals with increased inflammatory activation and neuroinflammation. These considerations prompted us to conduct a pilot study to examine whether CSF AChE and BChE activities were reduced in LLMD, and whether they were related to central and peripheral levels of pro-inflammatory cytokines IL-6 and IL-8. In addition, we examined if these indices of central cholinergic activity were related to CSF triggering receptor expressed on myeloid cells 2 (sTREM2) and CSF complement component 3 (C3), a potential specific marker of microglial activation and a marker of inflammation, respectively.

Methods

Participants

This study was approved by the institutional review boards of the Nathan Kline Institute for Psychiatric Research (NKI) and New York University School of Medicine (NYU SoM). Participants were volunteers who responded to advertisements in local newspapers and flyers or were recruited through the Memory Education and Research Initiative program run by the Geriatric Division at NKI. All participants provided formal informed consent prior to examination and were compensated up to $450.00 for completing the study.

A total of 133 participants completed baseline evaluation and 51 completed the optional lumbar puncture (LP). Of the 51 participants who performed the LP, three were excluded due to evidence of confluent deep or periventricular white matter hyperintensities (one or more hyper intense lesions measuring at least 10 mm in any direction) from magnetic resonance imaging (MRI), and one was excluded because of a Mini-Mental State Examination (MMSE) score below 28. Of the remaining 47 participants, 28 were diagnosed with Major Depressive Disorder (MDD) by a board-certified psychiatrist using the Structural Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV disorders (SCID) and 19 served as aged-match controls. Finally, CSF AChE and BChE levels were only determined in 27 depressed and 17 control participants. Of the 27 individuals with MDD, 20 (74%) had recurrent episodes. Please see table 1 for a summary of the demographic and clinical characteristics of the study participants.

Procedure

The study was conducted over 4 visits, usually 1 week apart. The first three visits were conducted at NKI and/or the Clinical and Translational Science Institute, NYU SOM. During the first visit, participants were given informed consent forms along with information on the study procedures and their rights. The participants' medical and psychiatric histories were collected and vital signs were measured. A psychiatric evaluation was then carried out while the MMSE was administered for global cognitive status. The Hamilton Depression Rating Scale (HAM-D) was administered to rate the severity of current depressive symptoms. Participants who met the criteria for past MDD but were not currently depressed (with a score less than 16 on the HAM-D) were included as MDD subjects. Lastly, blood was drawn for routine medical testing.

During the second visit, participants completed a Magnetic Resonance Imaging (MRI) scan of the head to quantify the magnitude of vascular brain pathology, which was used to rule out subjects with confluent deep or periventricular white matter hyper-intensities. During the third visit, participants underwent a comprehensive neuropsychological assessment. Lastly, during the fourth visit, an optional LP was performed by a neuroradiologist under guided fluoroscopy in those participants who consented. The LP required participants to fast overnight and was performed between 0900 and 1000 hours. A total of 15 mL of clear CSF was collected in three polypropylene tubes labeled A (first 5 mL), B (second 5 mL), and C (last 5 mL). The tubes were immediately placed on ice for a maximum of 1 h until samples were centrifuged at 4^{o}C at 1500 rpm for 10 min. Aliquots of 0.25 mL were then placed into 1 mL polypropylene cryogenic vials and stored in Nunc eight-cell storage boxes (Nalge Nunc Internation, Rochester, NY, USA) at -80^{o}C.

Protein and Cytokine Determination.

The lumbar puncture required participants to fast overnight and was performed between 0900 and 1000 hours. A total of 15 mL of clear CSF was collected in three polypropylene tubes labeled A (first 5 mL), B (second 5 mL), and C (last 5 mL). The tubes were immediately placed on ice for a maximum of 1 hour until samples were centrifuged at 4^{o}C at 1500 rpm for 10 min. Aliquots of 0.25 mL were then placed into 1 mL polypropylene cryogenic vials and stored in Nunc eight-cell storage boxes (Nalge Nunc Internation, Rochester, NY, USA) at -80^{o}C.

CSF and plasma levels of IL-6 and IL-8 were measured in a sandwich assay format using the Human Proinflammatory II 4-Plex Assay and a SECTOR instrument from Meso Scale Discovery Gaithersburg, MD. CSF AChE and BChE activities were determined using an in-house method as previously described (Parnetti et al., 2011). CSF sTREM2 concentration was measured using… The focus of this analysis will be on the baseline lumbar puncture determination.

Statistical Analysis

Nonparametric Mann-U Whitney tests were carried out to examine differences in CSF AChE and BChE between LLMD and controls. Spearman's correlations were then applied to examine associations between CSF AChE and BChE, and CSF (Il6, IL8, Abeta40, Abeta42, T-tau, Ptau, C3 and sTREM2) and plasma (IL-6 and IL-8) biomarkers. Statistical analysis was conducted in SPSS version 24.0. Figures were generated using Microsoft Excel 2016.

Results

CSF BChE levels were significantly lower in individuals with LLMD compared to controls ($z = 2.437$, $p = 0.015$), but no significant difference between groups was detected for CSF AChE levels ($Z = 1.556$, $p = 0.120$). This difference is reported in Figure 1.

Correlation coefficients and p values are reported in Tables 2 and 3. In LLMD, lower CSF BChE and AChE were associated with higher plasma IL-6. In contrast, in the control group, only lower CSF AChE was associated with higher plasma IL-8.

In both depressed individuals and controls, CSF BChE was associated with CSF C3. CSF BChE, in controls, was also positively associated with both CSF IL8 and CSF sTREM2. CSF AChE was associated with CSF sTREM2 and CSF P-tau in both individuals with LLMD and controls. In depressed individuals, we also observed a positive correlation between CSF AChE and

CSF Aβ40, whereas in controls positive correlations were detected between CSF AChE and both CSF Aβ42 and CSF T-tau.

Discussion

We found that the LLMD group had a significant reduction in CSF BChE compared with controls, but no significant difference in CSF AChE levels. We also found, that in the LLMD cohort, lower CSF AChE and BChE levels were associated with higher plasma IL-6, one of the pro-inflammatory cytokines frequently reported to be elevated in depression. In controls, lower CSF AChE was associated with higher plasma IL-8. In contrast, all the significant correlations that we observed between CSF BChE or AChE and CSF cytokines, C3 and sTREM2 were positive. Similarly, there were positive correlations with most of the CSF AD biomarkers (*i.e.*, Aβ40, T-tau, P-tau), whilst the correlation with CSF Aβ42 was marginally significant.

Acute inflammatory states associated with increased circulating peripheral inflammatory cytokines levels have been associated with reductions in plasma BChE, the major hydrolytic enzyme for ACh in the periphery, and lower levels have been associated with decreased cytokine release and better clinical outcome. These reductions have been interpreted as reflecting a compensatory activation of the cholinergic anti-inflammatory pathway. It is possible that the increased inflammatory signals from the periphery could activate the afferent vagus nerve fibers leading to the suppression of CSF proinflammatory markers via the release of ACh in depressed subjects. In this regard, a number of studies have shown a beneficial effect of vagus nerve stimulation in a number of inflammation-mediated disease models (Jarczyk et al., 2019). Further, we found that higher circulating IL-6 and IL-8 were associated with lower CSF AChE or BChE. Reductions in CSF AChE and BChE and brain AChE-mRNA have also been reported following acute intraperitoneal injection of bacterial lipopolysaccharide (LPS) in rats. Since α7 nAChR are also expressed in microglia and other brain inflammatory cells, any upregulation of the central cholinergic anti-inflammatory pathway, in response to increases in systemic inflammatory biomarkers, is likely to attenuate the neuroinflammatory response. Such a compensatory mechanism may play an important role in the regulation of excessive neuroinflammation in response to systemic cytokine activation in depressed individuals.

Reductions in neuroinflammation cause reductions in Aβ and tau production. Activation of the central cholinergic anti-inflammatory pathway is also known to shift microglia polarization from the M1 pro-inflammatory phenotype to the M2 associated with protective effects such as increased phagocytosis. Thus, these mechanisms may have contributed to lower CSF AD biomarkers which we observed in conjunction with lower CSF BChE and AChE levels.

In contrast to AChE, BChE is only expressed in a small number of cholinergic neurons and thus CSF BChE is less likely to reflect central synaptic cholinergic activity. It is also expressed in glial cells, including astrocytes and microglia, and may also be expressed in other inflammatory cells. In a study of patients with Alzheimer's disease (AD), increased CSF BChE was associated with increased glial fibrillary acidic protein (GFAP), a marker of astroglial activation. However, in a postmortem investigation in patients with multiple sclerosis, a disease characterized by neuroinflammation, BChE did not correlate with GFAP but it was associated with a marker of microglia activation. Both microglia and astrocytes are also known to express α7nAChRs whose

stimulation by ACh suppresses their activation. Thus, the significant CSF BChE reduction that we found in the LLMD cohort and its correlation with a number of inflammatory markers, and also with CSF AChE C3 which was observed only in this group, could simply reflect greater AChE-related suppression of brain inflammatory cells in this patient population. Studies using specific CSF biomarkers for astrocytes and microglia activation should be done to determine their relationship, if any, to CSF BChE and AChE level in LLMD. This will allow us to determine if the reduction in CSF BChE and the related correlations observed in LLMD group, reflect broad suppression of glia activation or more specific effects on microglia or astrocytes.

The role of BChE in inflammation is further strengthened by our findings of the association between BChE and the complement system. We found a significant positive correlation between CSF BChE and CSF C3 in individuals with LLMD and controls suggesting that CSF C3 levels may be regulated by cholinergic tone. C3 plays an important role in neuroinflammation and also in synaptic plasticity (Lee et al., 2019). Our recent study found a decrease in CSF C3 levels in LLMD subjects further strengthening the role of C3 in LLMD pathophysiology (Pillai et al., 2019). A number of previous studies have reported correlation of CSF C3 levels with the degree of neurological impairment in many neurodegenerative diseases (Wang et al., 2011). C3 is a member of the complement system family of more than 30 proteins which are regulated through three different activation pathways. However, being that C3 is the hub of all activation pathways, changes in C3 levels could impact the downstream mechanisms of complement activation leading to alterations in neuroinflammatory pathways. In particular, C3 has been shown to regulate microglial function and activity in neuroinflammatory conditions such as depression (Crider et al., 2018). Interestingly we also found that lower CSF AChE, which accounts for the hydrolysis of most of brain ACh and thus more likely to reflect cholinergic activity and suppression of brain inflammatory cell activation, was associated with lower CSF sTREM2 in both LLMD and controls. Several lines of evidence suggest that CSF sTREM2 may be a specific marker of microglia activation; thus, these correlations, if confirmed, implicate cholinergic activation in suppression of microglia activation.

To our knowledge, there are no studies which have examined the relationship between indices of cholinergic activity and inflammatory markers in depression. However, two studies, examined the relationship between the inflammatory cytokine IL-1b and other markers of inflammation or oxido/nitrosative stress in plasma or intracellularly in individuals with eating and personality disorders. They found that elevations in these indices were associated with increased expression of alpha 5 nicotinic receptor in peripheral blood mononuclear cells. ACh-mediated stimulation of these receptors is associated with a reduction in release of proinflammatory cytokines from inflammatory cells. These findings were interpreted as reflecting an upregulation of the cholinergic anti-inflammatory response; hence, they complement our results and provide support for the notion that changes in both central and peripheral indices involved in the cholinergic anti-inflammatory reflex may be important factors in the regulation of immune responses to increased inflammatory states in depression.

In preclinical and *in vitro* experiments, antidepressants have been shown to inhibit human BChE and AChE activity. Our analyses did not reveal a clear antidepressant effect on CSF BChE nor AChE. However, given the relatively small sample size, the possibility that antidepressant treatment might have contributed to the reduction in CSF BChE and upregulation of the cholinergic anti-inflammatory response in LLMD cannot be completely excluded.

The existing literature suggests that elevations in pro-inflammatory markers are not an invariant finding in depression. Only about 1/3 of depressed individuals have been reported to have

plasma cytokines higher than heathy controls. It is also not known how many of the depressed individuals with elevated plasma cytokine levels will have evidence of increased neuroinflammation. Studies using in vivo markers of neuroinflammation activation using various TSPO PET ligands, have provided conflicting data. Some studies, including a study in geriatric depression, have found evidence consistent with increased neuroinflammation, while others have reported no change and one found numerical reductions in diverse brain regions compared to controls. Indeed, both increased and decreased microglial activation have been implicated in the pathogenesis depression and different inflammation-based treatment approaches have been proposed for these distinct depression subtypes.

Our study has some limitations that are worth noting. Our sample size is relatively small, with slightly uneven distribution of LLMD and controls. Additionally, the level of severity of depression was different among the LLMD individuals. It is possible that the differences reported above may be further modulated by the degree of severity. The use and type of medication were also not controlled for in the LLMD group. Although the effect of medication was explored, these medications could have differentially affected the biological markers. Future studies should consider controlling for these important factors.

In conclusion, our preliminary results suggest that activation of the cholinergic anti-inflammatory pathway may be an important factor, which should be considered in determining if increased systemic inflammation associated with depression and other psychiatric disorders will impact neuroinflammation and brain function and thus etiologically contribute to the pathogenesis of these disorders. Interestingly acute administration of the antimuscarinic agent scopolamine has been found to be associated with rapid antidepressant response in treatment-resistant patients, and there is a large body of observations implicating excessive central cholinergic tone in the pathophysiology of depression. Thus, future studies should determine if upregulation of the cholinergic anti-inflammatory pathway could paradoxically contribute to treatment-resistant depression.

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Conflicts of interest

HZ has served at scientific advisory boards for Roche Diagnostics, Samumed, Wave and CogRx, has given lectures in symposia sponsored by Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

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