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Short Communication

Increased Contact System Activation in Mild Cognitive Impairment Patients with Impaired Short-Term Memory

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Abstract. An activated plasma contact system is an abnormality observed in many Alzheimer's disease (AD) patients. Since mild cognitive impairment (MCI) patients often develop AD, we analyzed the status of contact system activation in MCI patients. We found that kallikrein activity, high molecular weight kininogen cleavage, and bradykinin levels—measures of contact system activation—were significantly elevated in MCI patient plasma compared to plasma from age- and education-matched healthy individuals. Changes were more pronounced in MCI patients with impaired short-term recall memory, indicating the possible role of the contact system in early cognitive changes.

Keywords: Alzheimer's disease, bradykinin, contact activation, high molecular weight kininogen, memory impairment, mild cognitive impairment, plasma kallikrein

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder of multifactorial nature [1], and vascular factors are known to play an important role in its pathogenesis [2]. Cerebral amyloid angiopathy (CAA), or the deposition of the pathogenic amyloid-β (Aβ) protein in and around blood vessels, is a vascular abnormality present in 80–95% of AD patients [3]. Other cerebrovascular pathologies present in AD patients include cerebral blood flow alteration, blood-brain barrier (BBB) disruption, brain hypoperfusion, and leakage of blood proteins into the brain parenchyma [4–6]. For example, fibrinogen, a major blood protein required for clot formation, is extravasated in AD patient brains [7]. It has been shown that Aβ interacts with fibrinogen and alters blood clot structure and impairs clot degradation [8, 9]. Accumulated, persistent fibrinogen in the brain parenchyma induces inflammation and memory impairment [10]. Cerebral perfusion, BBB integrity, and cognition were improved in AD mice treated with dabigatran [11], which prevents fibrin clot formation, emphasizing the involvement of blood proteins in AD pathology [12]. Similar to this study, it has been shown that inhibiting the Aβ-fibrinogen interaction prevents AD pathology in mouse models [13]. Therefore, the interaction between Aβ and fibrinogen may exacerbate cerebral pathologies in AD patients.

The intrinsic blood coagulation pathway is triggered upon activation of factor XII (FXII) of the plasma contact system [14]. In addition to thrombosis, this pathway can promote an inflammatory

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response. When FXII is activated, kallikrein cleaves high molecular weight kininogen (HK) which thereby releases bradykinin [15, 16]. Aβ aggregates can trigger the contact system by activating FXII [17–19], thus enhancing both of these inflammatory and thrombotic pathways [14, 20–22].

Many AD patient plasma samples show increased levels of activated FXII, kallikrein activity, and HK cleavage [21, 22]. An increase in cleaved HK is positively correlated with dementia and neuritic plaque scores of AD patients [21]. Though contact system activation is not specific to AD [15], this finding indicates that a dysregulated contact system could affect AD pathogenesis and cognitive decline. Furthermore, knockdown of the contact system reduces cerebral inflammation, prevents fibrin extravasation, and improves cognition in AD mice [23].

There is increasing evidence that subtle losses in cognitive function may be an early indication of AD development. Mild cognitive impairment (MCI) refers to the transitional stage between the cognitive decline associated with normal aging and mild dementia [24, 25]. MCI patients perform reasonably well on indices of general cognitive function and their ability to carry out daily living activities is largely preserved, yet they do present with other acquired cognitive deficits, such as retrieval of episodic and short-term memory [24]. Many MCI patients progress to AD at a rate of 10% to 15% per year, while healthy control subjects are diagnosed with AD at a rate of 1% to 2% per year [24, 26, 27]. MCI patients often show gray matter loss and synaptic alterations [24, 28, 29], and MCI patients who subsequently progress to AD show hypoperfusion in the posterior cingulate cortex [24, 29, 30]. Furthermore, both MCI and AD patients present with cortical hypometabolism with some regional variability [24, 31, 32]. These findings support the belief that MCI is a risk factor for AD, and, as evidenced by imaging techniques, MCI and AD pathologies share many of the same structural and functional abnormalities.

A common biomarker for MCI and AD could be used to quickly diagnose MCI but would also provide an opportunity to identify those patients progressing to early-stage AD, which would allow for preventive care before severe AD onset. It has been reported that there is a significant alteration in the plasma protein profile of MCI patients who later develop AD, suggesting that plasma proteins could serve as a biomarker for MCI and AD [33, 34].

Here, we analyzed plasma samples from MCI patients and age-matched cognitively normal (CN) individuals and compared the status of contact system activation in both groups. We measured plasma bradykinin level, kallikrein activity, and HK cleavage. Our analysis suggests that the contact system is activated in MCI patients and the extent of activation correlates with impaired short-term recall memory. Our data support the early involvement of an impaired peripheral contact system in MCI and other neurodegenerative diseases.

MATERIALS AND METHODS

Plasma samples

Experiments using human plasma samples were reviewed and approved by The Rockefeller Institutional Review Board. Plasma from MCI patients and age-matched and education-matched CN individuals were obtained from PrecisionMed Inc. (San Diego, CA). Patients were screened for MCI using criteria developed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [35]. MCI diagnosis was established by clinical examination, including the Mini-Mental State Examination (MMSE) and other neuropsychological tests (Clinical Dementia Rating, CDR; Logical Memory test II, LM II; Alzheimer's Disease Assessment Scale Cognitive subscale, ADAS-Cog) [36]. All MCI patients had Hachinski score $\leq$4, indicating no multi-infarcts or vascular dementia [37]. None of the patients whose plasma was included in our study had history of stroke, heart attack, hypertension, hyperlipidemia, diabetes, rheumatoid arthritis, thyroid disease, or pernicious anemia. Other neurological conditions, such as chronic nervous system infection, Parkinson's disease, Huntington's disease, and Creutzfeldt–Jakob disease were also absent in this patient cohort. Brain MRI/CT images collected within two years of plasma donation were analyzed to exclude other possible causes of cognitive impairment. Subjects were not on anticoagulant therapy, non-steroidal anti-inflammatory medicine, or aspirin within a week of their visit for blood donation. The blood was collected in K2EDTA anticoagulant. The characteristics of MCI and CN individuals are presented in Table 1.

Plasma bradykinin level and kallikrein activity

Plasma bradykinin level was analyzed by ELISA as described previously [20, 38]. Plasma kallikrein

Table 1
Characteristics and demographics of mild cognitive impairment and cognitively normal individuals

Characteristics	Cognitively normal controls	Mild cognitive impairment patients	*p*
Individuals, *n*	19	25	
Ethnicity	Caucasian	Caucasian	
Female, *n*	10 (53%)	16 (64%)	
Male, *n*	9 (47%)	9 (36%)	
Hachinski score	–	≤4	
Mini-Mental State Examination (MMSE) score, mean (SD)	29.84 (0.37)	25.8 (1.95)	$p < 0.0001$
Registration memory score in MMSE, mean (SD)	3.0 (0)	2.64 (0.90)	$p = 0.092$
Recall memory score in MMSE, mean (SD)	2.89 (0.31)	0.92 (0.95)	$p < 0.0001$
Clinical Dementia Rating score, mean (SD)	–	0.52 (0.1)	
Alzheimer's Disease Assessment Scale Cognitive subscale score, mean (SD)	–	15 (7.6)	
Logical memory II score, mean (SD)	–	12.2 (8.7)	
Total years of education, mean (SD)	15.59 (1.62)	14.68 (1.81)	$p = 0.10$
Age (y) at blood draw, mean (SD)	65.79 (5.0)	65.12 (7.0)	$p = 0.72$
Age (y) at diagnosis, mean (SD)	–	62.83 (7.37)	
Disease duration (y), mean (SD)	–	1.95 (2.33)	
Stroke, hypertension, and heart attack status	Not present	Not present	
Diabetes, hyperlipidemia, and rheumatoid arthritis status	Not present	Not present	

activity was measured as described in [22] with some modifications. Briefly, in a 96-well plate, plasma samples diluted (1:20) in HEPES-buffered saline (20 mM HEPES, pH 7.4, 140 mM NaCl) were mixed with a chromogenic substrate, S-2302 (0.67 mM final concentration). Absorbance at 405 nm was read for 60 min at 37°C using a spectrophotometer (Molecular Devices). Samples were run in duplicate.

Plasma cleaved HK level and C1 esterase inhibitor (C1INH) level

The level of plasma cleaved HK was determined using a sandwich ELISA [21]. The monoclonal antibody (4B12) used in this ELISA specifically detects cleaved HK [21]. Plasma was diluted (1:50) in blocking buffer (1% bovine serum albumin in 0.1% tween-20/PBS), and ELISAs were performed in duplicate as described in [21]. Plasma C1INH level was quantified by ELISA (Abcam) as per the manufacturer's instructions.

RESULTS AND DISCUSSION

Plasma contact system is activated in MCI patients

The contact system is activated in AD patient plasma and correlates with the severity of memory impairment [21, 22]. Increased plasma bradykinin level, kallikrein activity, and HK cleavage are indicators of an activated contact system. Here, we analyzed the bradykinin level in plasma samples from MCI patients and age-matched CN individuals. We found that plasma bradykinin level was significantly increased in MCI patients compared to CN individuals (1590 ± 261.6 pg/mL versus 967.5 ± 109 pg/mL; $p < 0.05$). Since bradykinin is generated from HK by active kallikrein [15], we measured the plasma kallikrein activity in MCI and CN groups. We found that plasma kallikrein activity was also significantly elevated in MCI patients compared to that of CN (0.11 ± 0.01 versus 0.05 ± 0.009; $p < 0.01$). Kallikrein activity was abolished in MCI plasma when aprotinin, a known kallikrein inhibitor [39], was added to samples (Supplementary Figure 1A).

Analysis of MMSE data from MCI patients revealed that many MCI patients performed poorly on recall memory (Table 1). Recall memory is evaluated by giving the test subject a list of three unrelated words, and the subject is asked to recall them after several minutes. The maximum score for recalling three unrelated words in MMSE is 3 [40]. Many MCI patients (11/25) did not recall any of the words (score 0), indicating they had impaired short-term recall memory. Some patients (6/25) recalled one word (score 1). The plasma kallikrein activity of MCI patients with impaired recall (score 0 or 1) was significantly higher than that of CN individuals (0.12 ± 0.01 versus 0.05 ± 0.009; $p < 0.01$; Fig. 1A). Plasma kallikrein activity was higher in MCI patients with impaired recall (score 0 or 1) compared to that of MCI patients without impaired recall (8/25; score

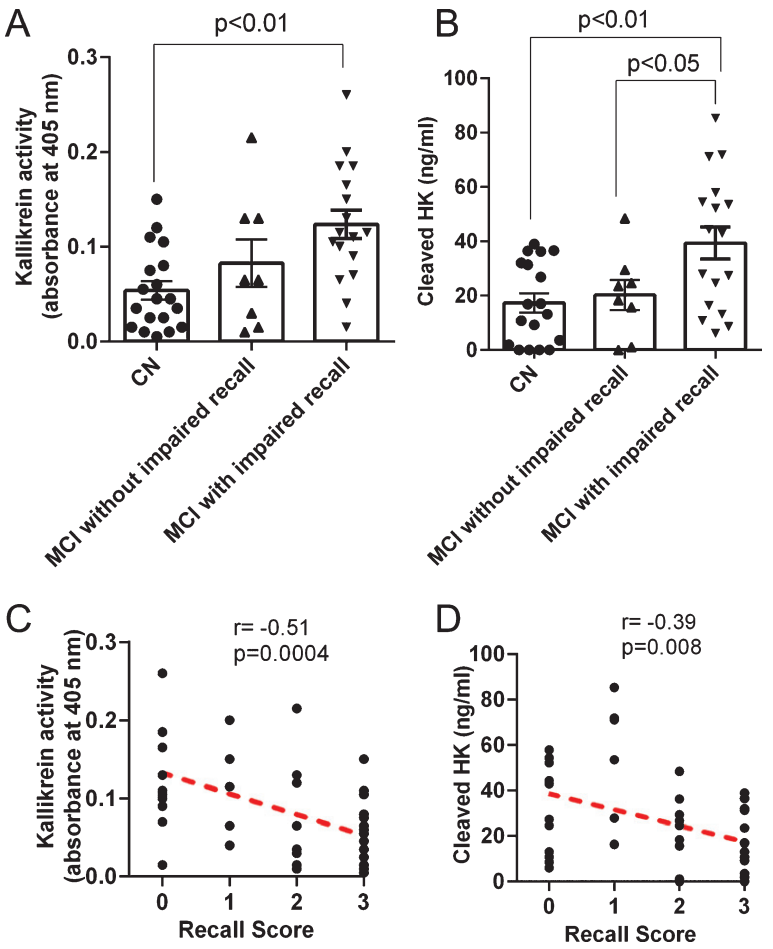

Fig. 1. Activation of contact system in mild cognitive impairment (MCI) patient plasma. Plasma kallikrein activity and cleaved HK levels in plasma from MCI and cognitively normal (CN) subjects were assessed using chromogenic assay and ELISA, respectively. MCI patients with low recall scores (0 or 1) were grouped as 'MCI with impaired recall'. MCI patients with higher recall scores (2 or 3) were grouped as 'MCI without impaired recall'. A) Plasma kallikrein activity was significantly higher in MCI patients with impaired recall memory compared to that of CN. B) Plasma cleaved HK levels were significantly higher in MCI patients with impaired recall compared to CN. C) Plasma kallikrein activity inversely correlates with recall score. D) Plasma cleaved HK level also inversely correlates with recall score. Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test. Correlation was analyzed using Pearson's correlation coefficient (r). Results are presented as mean ± SEM. N = 19 CN, 25 MCI. The difference in cleaved HK level between MCI without impaired recall and MCI with impaired recall was significant by t-test with Welch's correction.

2 or 3), though this trend did not reach significance (Fig. 1A).

We also quantified the level of cleaved HK in each patient's plasma sample, using a sandwich ELISA that differentiates between full-length HK and cleaved HK [21]. Similar to the kallikrein activity results, the ELISA showed that the level of cleaved HK in MCI patients with impaired recall (score 0 or 1) was significantly higher than that of CN individuals (39.31 ± 5.9 ng/mL versus 20.25 ± 4.4 ng/mL; $p < 0.01$; Fig. 1B). Cleaved HK levels in plasma from MCI patients with impaired recall were also higher than MCI patients without impaired recall (39.31 ± 5.9 ng/mL versus 20.15 ± 5.5 ng/mL; $p < 0.05$ Fig. 1B). This difference was significant when analyzed by an unpaired t-test with Welch's correction but not by one-way ANOVA (Fig. 1B).

The plasma protease, C1INH, negatively regulates contact activation, and a decrease in C1INH levels can trigger kallikrein generation and HK cleavage [15]. It has been reported that the level of plasma C1INH is reduced in MCI patients [41]. However, we did not find any significant difference in plasma C1INH levels between MCI and CN samples (59.9 ± 6.1 μg/mL versus 49.7 ± 7.1 μg/mL; $p = 0.28$; Supplementary Figure 1B). Therefore, the

increase in contact activation could be due to other mechanisms such as increased plasma Aβ [42], which can activate the contact system [17, 18, 22].

Episodic memory is the ability to recall events that are specific to a time and place [24]. Community-based longitudinal studies have found that the deficits in episodic memory can be found at least five years before the onset of clinical dementia [24, 43]. Episodic memory was also found compromised in MCI patients and linked with hippocampal atrophy [24].

In our patient cohort, no significant differences in memory registration (encoding) were found between MCI and CN groups (Table 1). However, recall memory was significantly impaired in MCI patients compared to CN individuals (Table 1). The recall memory score showed a significant inverse correlation between plasma kallikrein activity and plasma cleaved HK (Fig. 1C, D). Recall memory impairment has been also shown to correlate with synaptic alterations and gray matter loss in MCI patients [28, 29]. The posterior cingulate cortex, which contains the neural pathway for recall memory, is metabolically impaired in MCI patients. [24, 29, 44]. Peripheral changes, such as plasma contact system activation, may affect the central nervous system in such ways that ultimately affect recall memory in addition to blood coagulation and inflammatory conditions. For example, it has been shown that cerebral injection of bradykinin in rats affects memory [45]. Since bradykinin has been shown to impair the BBB [46], blood proteins may enter the brain parenchyma and lead to inflammatory processes, cell death, and memory loss. These mechanisms must be explored in more detail.

Peripheral protein and amino acid alterations are associated with cognitive function in MCI patients [33, 34, 41, 47, 48], and peripheral contact activation also correlates well with dementia in AD patients [21, 22]. In this study, we determined that the contact system is also activated in MCI patients and correlates with their recall memory status. A longitudinal study is warranted to evaluate how the contact system fully affects the progression from cognitively normal to stages of MCI and AD.

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SUPPLEMENTARY MATERIAL

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