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Neutrophil gelatinase-associated lipocalin: A novel inflammatory marker associated with late-life depression

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ABSTRACT

Objective: Systemic low graded inflammation has been identified as a possible biological pathway in late-life depression. Identification of inflammatory markers and their association with characteristics of depression is essential with the aim to improve diagnosis and therapeutic approaches. This study examines the determinants of plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL), which is selectively triggered by TNFα receptor 1 signaling within the central nervous system, and its association with late-life depressive disorder.
Methods: Baseline data were obtained from a well-characterized prospective cohort study of 350 depressed and 129 non-depressed older persons (≥60 years). Past 6 month diagnosis of major depressive disorder (MDD) according to DSM-IV-TR criteria was assessed with the Composite International Diagnostic Interview (CIDI 2.0). Potential determinants of plasma NGAL included sociodemographic characteristics, lifestyle and psychiatric and physical comorbidity.
Results: Plasma NGAL concentrations were significantly associated with age, male gender, smoking and waist circumference. Adjusted for these determinants, depressed patients had significantly higher NGAL plasma levels compared to non-depressed comparison group. Depressed patients who did not meet full criteria for MDD in the month before sampling (partially remitted) had lower plasma NGAL levels compared with those who did. Subjects with a recurrent depression had higher plasma NGAL levels compared to those with a first episode. NGAL levels were neither related with specific symptom profiles of depression nor with antidepressant drug use.
Conclusion: Adjusted for confounders, NGAL plasma levels are increased in depressed older persons, without any effect of antidepressant medication and age of onset.

Introduction

Immune dysregulation in depression has attracted a great deal of attention during the last two decades, since first proposed by Smith [1]. Two meta-analyses confirmed upregulation of peripheral inflammatory markers in depressed patients [2,3], most consistently for interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) [4–6]. Although data are scarce, increased expression of pro-inflammatory cytokines has been demonstrated in post mortem brain tissue from depressed patients [7,8], reflecting the anomalies found in the periphery. Recently, our research group identified Neutrophil Gelatinase-Associated Lipocalin (NGAL), a 25 kDa protein also known as Lipocalin-2, siderocalin, 24p3, or uterocalin [9], as a novel neuroinflammatory marker in patients with mild cognitive impairment and Alzheimer's disease [10].

Animal research has shown that NGAL is upregulated in the brain after the induction of a peripheral inflammatory response due to injection of an immunostimulant (LPS) [11] as well as after psychological stressors [12]. Interestingly, the increase of cerebral NGAL expression reduced hippocampal synaptic spine density [12]. Besides publishing the first results on NGAL in human brain tissue, we recently showed that NGAL expression is selectively triggered by TNF receptor 1 signaling [10]. In addition, we found that NGAL can induce a pro-apoptotic

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signaling cascade by attenuating Akt phosphorylation of the protein kinase B (PKB)/Akt pathway and sensitize neurons to beta-amyloid induced cell death [10]. Cellular signaling via Akt has been postulated as a key pathway involved in neuroplasticity in the hippocampus [13]. In vitro studies have illustrated the importance of Akt phosphorylation for neuronal survival and growth [14]. A recent study showed that PKB/Akt activity is also decreased in post mortem human brain tissue of suicide victims compared to non-depressed controls [15]. These data collectively suggest that increased central nervous system NGAL levels can attenuate Akt signaling that consequently can lead to reduced neuroplasticity. This hypothesized inhibitory effect of NGAL on hippocampal neuronal growth links NGAL to the "neurotrophic hypothesis of depression" [16]. Furthermore, increased hippocampal neurogenesis has been proposed as one of the underlying mechanism of antidepressants [17] and patients with late-onset depression have smaller hippocampal volumes [18]. NGAL may thus be an intriguing marker for future neurobiological and clinical research in late-life depression.

To our knowledge, this is the first study comparing NGAL plasma-levels between depressed and non-depressed older persons. Our objectives are 1) to explore determinants (and thus confounding factors) of plasma NGAL levels in humans, 2) to examine NGAL plasma levels as a biomarker for late-life depression, and 3) to explore which characteristics of the depression elucidate the hypothesized association with NGAL plasma levels.

Methods

Sample

The Netherlands Study of Depression in Older people (NESDO) [19] is an on-going cohort study designed to examine the (determinants of the) course and consequences of depressive disorders in older persons. Details of NESDO are described elsewhere [19], but can be summarized as follows. The NESDO sample consists of 378 depressed (major depression, dysthymia or minor depression according to DSM-IV criteria) and 132 non-depressed persons aged 60 through 93 years. Recruitment of depressed older persons took place in five regions in the Netherlands from both mental health care institutes and general practitioners. The ethical review boards of the participating institutes have approved this study. Persons with a primary diagnosis of dementia, a Mini Mental State Examination-score (MMSE) under 18 (out of 30 points), and insufficient command of the Dutch language were excluded. A non-depressed comparison group was recruited from general practitioners. Inclusion criteria for the non-depressed comparison group were: no lifetime diagnosis of depression, dementia or other serious psychiatric disorders, and good command of the Dutch language. All participants gave informed consent after oral and written information about the study. Interviews were audio taped to control the quality of the data. Data collection of the first measurement started in 2007 and was finished in September 2010. The first measurement lasted 3 to 4 h and included written questionnaires, an interview, a medical examination, and collection of blood and instructions for the saliva samples. When necessary, the assessments were spread over two days. The present study uses data from this first measurement.

Depression diagnoses

Diagnoses of depression and dysthymia according to DSM-IV-R criteria (APA, 2000) are assessed with the Composite International Diagnostic Interview (CIDI; WHO version 2.1; 24 month version) [20]. The CIDI is a structured clinical interview that is designed for use in research settings and has high validity for depressive and anxiety disorders [21]. We added some questions to determine the research DSM-IV diagnosis of current minor depression [22]. For the present analyses, we selected the non-depressed persons (comparison group) and depressed patients who met the criteria for a major depressive episode within the last 6 months (MDD). The majority of these patients had a current diagnosis of depression, but some had a diagnosis of depression 1–6 months prior to baseline and did not fulfill all criteria in the past month. As this latter group had still significant depressive symptoms, it was classified as 'partially remitted'.

NGAL measurement in plasma by ELISA

Quantification of NGAL from plasma was performed via a constructed sandwich ELISA using human Lipocalin-2/NGAL ELISA capture antibody (R&D Systems), recombinant human Lipocalin-2/NGAL (R&D Systems) for the internal standard and biotinylated human Lipocalin-2/NGAL detection antibody (R&D Systems). Plasma was diluted 1:100. A blinded ELISA analysis was performed on coded samples. Briefly, plates (96 wells, Maxisorb, Nunc) were coated with the capture antibody (100 μl; 2 μg/ml) diluted in phosphate buffered saline (PBS, pH 7.4). After overnight incubation at room temperature, the coated plates were washed with Tris buffered saline (TBS) containing 0.05% Tween 20 (TBS/T) and nonspecific binding sites blocked by incubation with 300 μl of PBS containing 1% Bovine Serum Albumin (BSA) (PBS/BSA) for 2 h at room temperature on a shaker. After washing, 100 μl of either the standards (recombinant human lipocalin-2) or samples, diluted in PBS/BSA was added to the plates and incubated for 2 h at room temperature on a shaker. Plates were washed six times and 100 μl of biotinylated human Lipocalin-2/NGAL detection antibody (100 ng/ml) diluted in PBS/BSA was added. After 2 h on a shaker at room temperature, plates were washed six times and incubated with 100 μl Avidin-horseradish peroxidase (eBioscience) in PBS/BSA (1:1000) for 20 min on shaker at room temperature. Plates were then washed six times and 100 μl of substrate solution containing 1 mg/ml of O-phenylenediamine (Sigma) in 0.05 M citric acid sodium phosphate Buffer (pH 5.0) with hydrogen peroxide (0.06%) was added. The reaction was stopped by adding 100 μl of a 3 N HCl solution. The absorbance was determined at 492 nm with background subtraction at 620 nm using an ELISA reader (Asys UVM 340, Biochrom, Cambridge, UK). The intra- and inter-assay coefficients of variation were 2% and 5%, respectively. Samples were stored at −80 °C. Blood samples were collected in the morning to standardize for collection time [19].

Potential determinants of NGAL

The following variables were examined as potential determinants of NGAL plasma levels in humans. Demographic data were collected during the interview (age, gender, partner status and years of education). Lifestyle variables included smoking, alcohol uses, waist circumference and physical activity. Smoking was defined as currently smoking (yes/no). We assessed alcohol consumption with the Alcohol Use Disorders Identification Test (AUDIT), a questionnaire consisting of 10 items with a minimum score of 0 and a maximum score of 40 [23,24]. The total AUDIT score encompasses not only the volume and frequency of alcohol use, but also other drinking behavior risks. Physical activity was measured with the last-seven-days short-form (8-items) of the self-administered version of the International Physical Activities Questionnaire (IPAQ)[25]. Psychometric properties of the long and short version of the IPAQ are acceptable [25].

Global cognitive functioning was assessed by the Mini Mental State Examination (MMSE) [26]. The MMSE score ranges from 0 to 30, with higher scores indicating better cognitive functioning. The number of chronic diseases was assessed with previously used self-report questions about the presence of the following chronic diseases or disease events: cardiac disease (including myocardial infarction), peripheral atherosclerosis, stroke, diabetes mellitus, COPD (asthma, chronic bronchitis or pulmonary emphysema), arthritis (rheumatoid arthritis or osteoarthritis) and cancer. The accuracy of self-reports of these