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Short communication

Serum sortilin-derived propeptides concentrations are decreased in major depressive disorder patients

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ABSTRACT

Background: Despite intense research on mechanisms underlying the depressive pathophysiology, reliable biomarkers to assess antidepressant treatment response are still lacking. Since the sortilin-derived propeptide (PE) displays potent antidepressant activities and can be measured in the blood of rodents, we wondered whether in human its seric level can vary between patients affected by major depressive disorder (MDD) and healthy controls and after antidepressant treatment.

Methods: By using a specific dosing method, characterized by structure-recognition analysis with various synthesized PE analogues, we conducted a translational study to test whether blood levels of PE are under pathophysiological regulation and could serve as biomarkers of the depression state.

Results: The serum concentration of PE, a peptide displaying potent antidepressant activities in rodents, is decreased in patients affected by major depressive disorder (MDD) when compared to healthy non-psychiatric controls cohort (p=0.035). Interestingly, pharmacological antidepressant treatments restore normal PE levels.

Limitations: The limitation of the study concerns the relatively small patient samples that could negatively affect the likelihood that a nominally statistically significant finding actually reflects a true effect.

Conclusions: The longitudinal quantification of the serum PE concentration could assist psychiatrists in the diagnosis of antidepressant response efficacy, and the need to modify the therapeutic strategy.

1. Introduction

Major depressive disorder (MDD) episodes are treated with different drug classes. However any first-line of treatment currently leads, after several weeks, to a remission of about 35%, and approximately 30% of MDD patients are classified as having treatment resistant depression (TRD) (Bentley et al., 2014; Thomas et al., 2013; Wong and Licinio, 2001). Despite significant research efforts aimed at understanding the neurobiological underpinnings of MDD, treatments are still based solely on relatively subjective assessment of symptoms. Due to the low rate of remission, the identification of robust biological markers predicting the clinical evolution of MDD and characterizing the extent of the treatment outcome is therefore mandatory (Nestler et al., 2002). Clinical and preclinical studies have identified a number of factors that may serve as putative biomarkers for diagnosing and treating MDD. However, the utility of any given marker to serve as a clinically useful biomarker of MDD is limited by a lack of sensitivity and specificity (Jani et al., 2015; Schmidt et al., 2011).

Recent studies have pointed out the Brain Derived Neurotrophic Factor (BDNF) as a potential biomarker for clinical response under antidepressive pharmacotherapy and clinical outcome (Allen et al., 2015; Nase et al., 2016). Interestingly, sortilin is known to control intracellular sorting of BDNF to the regulated secretory pathway (Chen et al., 2005). Moreover, increased serum levels of sortilin are associated with MDD and correlated with BDNF (Belzeaux et al., 2010; Buttenschon et al., 2015). Recently, we have identified spadin, which is a partial peptide (12–28) of the 44 amino-acid propeptide (PE) generated from the maturation of sortilin (Munck Petersen et al., 1999), also called neurotensin receptor-3 (Mazella et al., 1998). When injected *iv* or *ip* in mice both spadin and PE display potent anti-

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depressant (AD) activities through inhibition of the potassium channel TREK-1 activity (Mazella et al., 2010), a target for depression treatment (Heurteaux et al., 2006). We originally developed a method to measure the PE level in mouse (Mazella et al., 2010). To undertake the dosing in human, we characterized the ability of the antibody directed against spadin to recognize peptides derived from the human sequence of PE. We demonstrated that both human peptides (PE and spadin) can be measured from human sera samples and we addressed the possibility that these peptides represent biological markers of MDD. Then, we measured the serum PE concentrations in a cohort of 37 patients with MDD treated with a pharmacological protocol and compared to 49 healthy non-psychiatric subjects.

2. Methods

2.1. Animals

All experiments were carried out on 20–25 g C57Bl/6 J males of 8–10 week old (Janvier France Breeding) according to policies on the care and use of laboratory animals of European Community legislation 2010/63/EU. The local Ethics Committee (CIEPAL) approved the protocols used in this study (protocol number 00893.02).

2.2. Antibodies and biotinylated peptide preparation

Peptides were synthesized by Genecust (Dudelange, Luxemburg). Rabbit polyclonal antibodies against spadin (YAPLPRWSGPIG-VSWGLR) were prepared by Eurogentec (Seraing, Belgium). Spadin (5.4 mg; 2.7 mmol) was solubilized in 1.5 mL of 25 mM phosphate buffer, pH 6.7. N-hydroxysuccinimide biotin (13.5 mmol) resuspended in 700 µl of 70% acetonitrile, 30% dimethyl formamide was added to the spadin solution and incubated overnight at room temperature. Spadin-biotinylated was purified by HPLC using a Waters apparatus equipped with a semi-preparative RP18 Lichrosorb column. Spadin-biotinylated (eluted at 27 min), identified by mass spectrometry, was collected, quantified by its absorption at 280 nm and lyophilized in aliquots.

2.3. Alpha-Lisa™ test

According to the principles of AlphaScreen™ technology (Perkin Elmer), streptavidin-donor microbeads were recognized by biotin-spadin, whereas anti-rabbit IgG-acceptor microbeads were bound by anti-spadin antibodies. When the two microbeads (acceptor and donor) were into proximity, the signal was produced by a molecular interaction occurring between the binding partners bound on the beads. The propeptide present in the serum sample was able to interfere with this interaction leading to competition. Standard curves were obtained by incubation in 96-well plates of 10 nM biotin-spadin with the anti-spadin antibody (1:1000) in the AlphaLisa™ buffer in the absence or in the presence of increasing concentrations of spadin (from 10^{-11} to 10^{-6} M) for 1 h at room temperature. After addition of acceptor and donor beads and further incubation for 2 h at room temperature, the plaques were read using the Enspire apparatus (Perkin). For serum measurements, the same volume of serum was added instead of unlabeled spadin. The amount of propeptide was determined from its percent of signal inhibition and calculated using the standard curve.

2.4. Porsolt forced swim test (FST)

After *iv* injection of either saline or various tested peptides, mice (n =10–12 per group) were placed individually in a cylinder (height: 30 cm, diameter: 15 cm) filled with water to a depth of 12 cm (temperature: 22 ± 1 °C) for 6 min. The total period of immobility was recorded during the last 4 min (Porsolt et al., 1977).

2.5. Human blood samples

The control cohort consisted of 49 unrelated healthy volunteers who were screened for DSM-IV Axis I disorder diagnoses by expert psychologists using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Only healthy volunteers without history of drug or alcohol abuse or dependence and without a personal or first-degree family history of psychiatric disorders were enrolled in the study. Furthermore, the absence of relevant neurological diseases *i.e.* epilepsy, Parkinson's syndrome, was mandatory for inclusion into the study. Finally, subjects who obtained a score lower than 27/30 at the Mini Mental State Examination (MMSE) were excluded from the study.

The patient cohort was made of 37 MDD patients with moderate to severe depression who met Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) classification system criteria. Diagnosis of unipolar depression was confirmed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). The exclusion criteria were: a) mental retardation or cognitive disorder; b) a lifetime history of schizophrenic, schizoaffective, or bipolar disorder; c) personality disorder, substance abuse, alcohol abuse or dependency, obsessive compulsive disorder, or post-traumatic stress disorder as the primary diagnosis; and d) comorbidity with an eating disorder.

No patients showed psychotic symptoms; 11 (29.7%) showed current comorbidity in Axis I (generalized anxiety disorder (GAD), panic attacks, panic disorders or anxiety disorder not otherwise specified (NOS)), 2 (5.4%) showed symptoms of Axis II disorders (dependent personality disorder) and no alcohol abuse, as a secondary diagnosis (the total number exceeded the number of subjects due to the presence of comorbidities).

All patients were either 'drug naïve', and had never received previous treatment with any antidepressant drug, or 'drug free'. They have been previously treated with one or two antidepressants but had a washout period lasting at least 2 weeks before starting with the new antidepressant treatment. All patients were treated in monotherapy: thirty-five patients were treated with selective serotonin reuptake inhibitors (SSRIs), and the other patients were treated with selective serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclics (TCAs) or noradrenergic and specific serotoninergic antidepressants (NASSAs).

Illness severity was assessed by the Montgomery and Asberg Depression Rating Scale (MADRS) before the start of the new antidepressant treatment (T0) and after 12 weeks of treatment (T1). All of the socio-demographical, clinical and pharmacological treatment characteristics of patients are shown in Table 1.

For both patients and controls, venous blood samples were collected between 8:00 and 9:00 a.m. after an overnight fast in anticoagulant-free tubes. Serum was separated by centrifugation (1620*g* for 15 min). Blood samples for PE measurements were collected at each timepoint.

Studies were approved by the Local Ethics Committees (CEIOC IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia N: 50/2008 and Ethics Committee of the province of Verona N: 4997/09.11.01), and written informed consent was obtained.

2.6. Statistics

Results are expressed as mean ± standard error mean (SEM). Statistical analyses were performed using GraphPad (version 6.0). Analysis of variance (ANOVA) was used to compute differences in time immobility for the different peptides used in the FST (Fig. 1D). Demographic and clinical characteristics in our patient samples were described either in quantitative term of mean ± standard deviation (SD) or as proportions. Chi-square ($\chi 2$) tests were conducted to evaluate the association between groups and categorical variables, while analysis of variance (ANOVA) was used to compute possible differences in age, education and BMI between groups (Table 1). Due to