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Plasma concentrations of oleoylethanolamide in a primary care sample of depressed patients are increased in those treated with selective serotonin reuptake inhibitor-type antidepressants.

Short title: Acylethanolamides and Depression

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Abstract

Oleoylethanolamide (OEA) is a non cannabinoid acylethanolamide with multiple physiological roles that has been proposed to have antidepressant-like activity in preclinical models. OEA shares biosynthetic pathways with anandamide (AEA) a transmitter involved in affective disorders and anxiety in humans. However, although the participation of OEA in depression has been proposed, both, the contribution of OEA to the depressive phenotype and the effect of antidepressant therapy on circulating levels of this and related non-cannabinoid acylethanolamides in humans are basically unknown. The main objective of this study is to compare the plasma concentrations of OEA and related acylethanolamides in a sample of primary care patients with depression (n=69) with those of healthy non-depressed patients (n =47). At the time of admission to the study, 22 patients were under selective serotonin reuptake inhibitor (SSRI) antidepressant treatment and 47 patients were not receiving any type of intervention. In addition, plasma concentrations of the endocannabinoid 2-AG and two related monoacylglycerols were monitored. Plasma OEA concentrations were found to be elevated in depressed patients and to correlate with somatic symptoms of depression. Plasma concentrations of both, AEA and 2-AG, were found to be elevated also in depressed patients. Further analysis demonstrated that the elevation observed in the plasma concentrations of both, OEA and 2-AG, was associated to SSRI antidepressant therapy at the time of recruitment. Further clinical research is needed to understand whether SSRI-induced elevations in OEA levels contribute to the response to SSRI in depressed patients as described in preclinical models.

Key words

Acylethanolamides; Anandamide; Antidepressants; Depression; Endocannabinoids; Oleoylethanolamide; Primary Care

1. Introduction

Depression is the fourth-leading cause of disease burden. Some reports have stated that 25% of the population will experience depression symptoms at some point in their lives, being the illness that provokes the second highest level of disability worldwide (Mathers and Loncar, 2006; Richards, 2011). Improving the diagnosis and treatment of depression is a challenge for all mental health care professionals and researchers, given its substantial personal, economic and social costs (Greenberg et al., 2003; Sobocki et al., 2006). Traditionally, pharmacological treatment of depression has been focused on increasing monoamines in different brain areas by using drugs that prevent their reuptake, such as serotonin-selective reuptake inhibitors (SSRIs) or noradrenaline-selective reuptake inhibitors (NSRIs). However, this pharmacological approach is not effective for all patients (Coppola and Mondola, 2014). This issue has led to the search for other pharmacological strategies addressing innovative targets within a broader conceptualization that comprises the body's response to stress (Slavich and Irwin, 2014; Zajkowska et al., 2014). Following this rationale, in the last decade much effort has been developed to study the contribution of the endogenous cannabinoid system (ECS) to the pathogenesis and therapy of stress-associated disorders, including depression (Gorzalka et al., 2008; Hill et al., 2009; Rubino et al. 2015).

The endocannabinoid system (ECS), is an on-demand lipid transmitter-based modulatory system regulating multiple functions in the brain and peripheral tissues (Piomelli et al., 2003; Mechoulam and Parker, 2013). The ECS includes two main chemical species, acylethanolamides (such as arachidonylethanolamide [AEA]) and monoacylglycerols (such as 2-arachidonoylglycerol [2-AG]). Both, AEA and 2-AG engage with cannabinoid CB1 and CB2 receptors located in neurons and glial cells to control synaptic transmission in circuits involved in important homeostatic functions including eating, reproduction, social behaviour, playing, learning, memory and stress responses (Mechoulam and Parker, 2013; Wei et al., 2017). In addition to these cannabinoid receptor-signaling lipids, other molecular congeners devoid of cannabimimetic properties have been described. Among them, the satiety factor oleoylethanolamide [OEA]) that exerts its actions through the peroxisome proliferators receptor alpha (Rodríguez de Fonseca et al., 2001; Fu et al., 2003) has gained much relevance for its role in mediating hedonic responses for food and drugs (Tellez et al., 2013; Bilbao et al., 2013 and 2016).

Concerning endocannabinoids, both, preclinical and clinical studies have demonstrated a link in between anandamide and 2-AG and affective disorders (Hill et al., 2008; Blankman and Cravatt, 2013; Mechoulam and Parker, 2013; Hillard and Liu, 2014). According to these studies, a decrease in endocannabinoids results in the presence of behaviours that mimic the symptoms of human depression, whereas an increase in extracellular endocannabinoids resulted in antidepressant effect. This finding originated from several studies in animals, that include pharmacological strategies to reduce endocannabinoid degradation using selective blockers of the main endocannabinoid-hydrolyzing enzyme fatty acid amide hydrolase (FAAH) (Khaturia et al., 2002; Blankman and Cravatt, 2013; Hillard and Liu, 2014). They were also confirmed in human observations (Hill et al., 2008 and 2009). Regarding non-cannabinoid acylethanolamides, such as palmitylethanolamide (PEA) or oleylethanolamide (OEA), its contribution to human affective disorders is much less known, although there is substantial evidence coming from preclinical experimental reports. Thus, pharmacological administration of OEA was found to display antidepressant activity in animal models of depression (Jin et al., 2015) through the modulation of serotonin/noradrenaline release (You et al., 2015). This effect was attributed to the interaction of OEA with peroxisome proliferator-activated receptors- α (PPAR α) receptors, since it was mimicked by clinically-used PPAR α agonists such as fenofibrate (Jiang et al., 2017), potentially by activating central PPAR α receptors present in hippocampal cells (Song et al., 2018). In addition, OEA was found to ameliorate depressive symptoms associated to either lipopolysaccharides (LPS) administration (Sayd et al., 2014) or to acute alcohol exposure (Antón et al., 2016), helping to reduce LPS release from the intestine into the circulation by preventing alcohol-inducing damage (Anton et al., 2018). The neurobiological basis of the potential antidepressant actions of OEA also involves both Histamine (Provensi et al., 2014; Costa et al., 2018), and Oxytocin (Gaetani et al., 2010) circuits, which are involved not only in the feeding suppression induced by this acylethanolamide but also in the antidepressant actions of SSRIs, the main class of antidepressants used for the therapeutics of depression (Uvnäs-Moberg et al., 1999; Munari et al., 2015; Scantamburlo et al., 2015).

Given the previous findings, and the lack of studies addressing the effect of SSRI on circulating OEA levels in humans, the main aim of this study was to compare the concentrations of oleylethanolamide and related congeners in a sample of patients with depression demanding treatment. Patients were recruited in a primary care setting, and the effect of SSRI, the influence

of the severity of depression, the type of symptoms (cognitive affective vs. somatic), age, sex and BMI were analyzed.

2. Methods

2.1 Participants and recruitment

Two samples were used in this study. The clinical sample consisted of 69 patients with mild or moderate depression. The participants were recruited in primary care settings by general practitioners from the population of patients who consulted for mood disturbances and who participated in another research project about psychotherapy for depression. The inclusion criteria were the following: a) age between 18 and 65 years; b) depressive symptoms for over two months; c) diagnosis of major depressive disorder; and d) signed informed consent. The exclusion criteria were the following: a) diagnosis of a severe mental disorder or a substance use disorder; b) current pregnancy or breast-feeding; and c) presence of chronic infectious or inflammatory diseases. Patients were evaluated by clinical psychologists. Their mean age was 43.23 years ($SD=9.64$), 71% were female, and 71% were married or had a stable partner. All patients were Caucasian. In order to identify the effects of SSRI-type antidepressant therapy on OEA levels, current SSRI treatment was not considered exclusion criteria. Thus, the final sample included 47 depressed patients without any active intervention and 22 with active SSRI pharmacotherapy. The mean duration of the antidepressant treatment was 179.77 days ($SD=126.87$). In addition, one participant used anti-inflammatories, four antihypertensives and 24 anxiolytics.

The control group was composed of 47 healthy volunteers from the hospital staff who were recruited by the researchers. All participants were briefly interviewed using a screening interview to rule out the presence of psychopathology, substance use disorder, psychotropic medication use or any other medication in the last month. Their mean age was 39.55 years ($SD=11.55$), 66% were female, and 48.9% were married or had a stable partner. All participants from the control group were Caucasian. Participants in the control group were selected to match on age, gender, and sex with the patients in the depressive group.

2.2 Ethics statement

Written informed consent was obtained from each subject after they had received a complete description of the study and been given the chance to discuss any questions or issues. The study and protocols for recruitment were approved by the Regional Research and Ethics Committee of the Hospital Regional Universitario de Málaga and were therefore conducted in accordance with the 'Ethical Principles for Medical Research Involving Human Subjects' adopted in the Declaration of Helsinki by the World Medical Association.

2.3 Measures

Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996).

This inventory consists of 21 multiple-choice items that assess typical symptoms of depression. The BDI-II has demonstrated excellent internal consistency, validity, and test-retest reliability (Beck, et al., 196a and 1996b). In this study, the BDI-II total score was used as a measure of the severity of depression (mild: 14-19; moderate: 20-28). Cognitive-affective and somatic factors found by Beck et al. (1996b) were used as measures of the severity of cognitive-affective (e.g. sadness, concentration difficulty or indecisiveness) and somatic symptoms (e.g. fatigue, appetite change, sleep changes or decreased libido).

Structured Clinical Interview for DSM-IV Axis I Disorders– Clinician Version. (SCID-CV)

The SCID (First et al., 1997) is a semi-structured interview that assesses Axis I disorders from the fourth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). It is internationally widespread, and it is considered the gold standard instrument for the diagnosis of DSM-IV disorders. In this study, the SCID-CV was used to standardize the major depressive disorder diagnoses.

2.4 Blood extractions

Blood extractions were conducted prior to interviews in 12-h-fasted conditions and in the morning (9:00– 11:00 am). Venous blood samples were extracted into 10-ml K2 EDTA tubes (BD, Franklin Lakes, NJ, USA) and to obtain plasma, samples were immediately centrifuged at 2200 g for 15 minutes (4°C). Plasma samples were individually assayed by three rapid tests to detect

infectious diseases: HIV (Retroscreen HIV, QualPro Diagnostics-Tulip Group Ltd, Goa, India), hepatitis B (HBsAg Test, Toyo Diagnostics- Turklab Inc., Izmir, Turkey) and hepatitis C (Flaviscreen HCV, QualPro Diagnostics-Tulip Group Ltd). Each plasma sample was registered and characterized, discarding the samples that displayed infection following safety protocols. All samples were stored at -80°C until the acylethanolamides and monoacylglycerols were quantified.

Quantification of acylethanolamides and monoacylglycerols

The following acylethanolamides were quantified: the monounsaturated oleoylethanolamide (OEA) and Palmitoleoylethanolamide (POEA), and the polyunsaturated arachidonoyl ethanolamide (AEA), dihomo- γ -linoleoylethanolamide (DGLA), docosatetraenoylethanolamide (DEA), linoleoylethanolamide (LEA), docosahexaenoylethanolamide (DHEA). The monoacylglycerols monitored were 2-arachidonoylglycerol (2-AG), 2-linoleoylglycerol (2-LG), and 2-oleoylglycerol (2-OG). Briefly, aliquots of 0.5 mL of human plasma were transferred to 12 mL glass tubes, spiked with deuterated internal standards, diluted with 0.1 M ammonium acetate buffer (pH 4.0), and extracted with *tert*-butyl methyl ether. The dry organic extracts were reconstituted in 100 μL of a mixture water: acetonitrile (10:90, v/v) with 0.1% formic acid (v/v) and transferred to HPLC vials. Twenty μL were injected into the liquid chromatography-coupled tandem mass spectrometry system. An Agilent 6410 triple quadrupole (Agilent Technologies, Wilmington, DE, USA) equipped with a 1200 series binary pump, a column oven and a cooled autosampler (4°C) was used. Chromatographic separation was performed with a Waters C18-CSH column (3.1 x 100 mm, 1.8 μm particle size) maintained at 40°C with a mobile phase flow rate of 0.4 mL/min. The composition of the mobile phase was: A: 0.1% (v/v) formic acid in water and B: 0.1% (v/v) formic acid in acetonitrile. The mass spectrometry analysis was performed on the single reaction monitoring mode (SRM). Quantification was performed by isotope dilution. The deuterated internal standards were obtained from Cayman Chemical (Ann Arbor, MI, USA), and the solvents were from Merck (Darmstadt, Germany). The entire procedure can be consulted at Pastor et al.(2014) and has been previously validated in clinical samples (Pavon et al., 2013)

2.5 Statistical analysis and strategic planning

The normality of the scores was determined using Shapiro-Wilk tests. Differences in the data expressed in percentages were tested using Chi-Square tests. For data expressed by means

and standards deviations (SD), differences between groups were tested with Student's t-tests or Mann-Whitney's U tests, depending on the normality of the scores. Correlations were calculated using Pearson's R for continuous and normally distributed data. Otherwise, Spearman's Rho (r_s) was used. Although multiple comparisons were performed, Bonferroni correction was not applied, given the exploratory nature of the study. In addition, receiver operating characteristic (ROC) analyses considering the area under the curve (AUC) were used to identify predictors for discriminating groups and to evaluate the predictive power of the logistic models. Binary logistic regression models were generated including the selected predictors, and the goodness of fit of the model was tested with the Hosmer-Lemeshow test. A backward stepwise approach was used to restrict the model to the most predictive predictors. A p value less than 0.05 was considered statistically significant.

Statistical analyses were performed using Graph-PadPrism version 6.01 (GraphPad Software, San Diego, CA, USA), whereas the logistic regression models were performed using IBM SPSS Statistical version 22.0 (IBM, Armonk, NY, USA).

3. Results

3.1 Preliminary analyses

The socio demographic characteristics of the patient/control groups are presented in Table 1. The results showed that both samples were similar in age, gender and BMI. The analyses also showed statistically significant differences between samples in marital status ($p < 0.01$), education ($p < 0.05$) and employment status ($p < 0.001$). Patients with depression were more often married, had less university studies and were more often unemployed than the control group. The results regarding the severity of depression and the pharmacological treatments are presented for the clinical sample only.

//Insert Table 1//

3.2 Differences in acylethanolamides and acylglycerols between depressed patients and controls

The Shapiro-Wilk test showed that the distributions of most of the lipids measured were not normal; therefore, Mann-Whitney's U was chosen to test the differences between samples in the analyses in this section and the next. Depressed patients showed significantly higher concentrations of mono-unsaturated [OEA ($U=892.000$; $p<0.001$) and POEA ($U=1124.000$; $p<0.01$)] acylethanolamides, as well as those of the poly-unsaturated ones [AEA ($U=969.000$; $p<0.001$), LEA ($U=987.000$; $p<0.001$), DEA ($U=1074.000$; $p<0.01$), DGLEA ($U=1037.000$; $p<0.001$), and DHEA ($U=1218.000$; $p<0.05$)] (See Figure S1). The analysis of the monoacylglycerols showed that depressed patients had a statistically significantly higher concentration of 2-AG than controls ($U=946.000$; $p<0.001$). The analyses did not show statistically significant differences in the other monoacylglycerols, 2-LG or 2-OG. See Figure S2. All results remained significant after applying Bonferroni correction, except for those of DHEA and POEA.

//Insert Figure 1 and Figure 2//

3.3 Effects of sex, age and body mass index, on plasma concentrations of acylethanolamides and acylglycerols in both depressive and control groups.

As the Shapiro-Wilk test showed that most of the variables were not normally distributed, Spearman's Rho was chosen to test the correlation between acylethanolamides and monoacylglycerols and sex, age and body mass index. The analyses showed no statistically significant differences between sexes in the plasma concentration of these bioactive lipids in the depressive sample. Only in the control group, we found that the concentration of DHEA was significantly higher in men ($U=144.000$; $p<0.05$) than in women. (See supplementary material, tables S1 to and S4).

Regarding age, in the clinical sample, 2-AG ($r_s=0.254$; $p<0.05$), 2-OG ($r_s=0.265$; $p<0.05$), DHEA ($r_s=0.369$; $p<0.01$), and OEA ($r_s=0.347$; $p<0.01$) were positively correlated with age, whereas DEA ($r_s=-0.319$; $p<0.01$) was negatively correlated with age. In the control sample, again 2-AG ($r_s=0.371$; $p<0.01$) and 2-OG ($r_s=0.461$; $p<0.001$) were positively correlated with age, whereas DEA ($r_s=-0.402$; $p<0.01$) and LEA ($r_s=-0.358$; $p<0.05$) were negatively correlated. All

correlation analyses are presented as supplementary tables (see tables S5 and S6). Only 2-AG ($r_s = 0.257$; $p < 0.05$) and 2-OG ($r_s = 0.241$; $p < 0.05$) showed statistically significant positive correlations with BMI in the clinical sample, and only DGLEA ($r_s = 0.297$; $p < 0.05$) was significant in the non-clinical sample (See tables S7 and S8).

//Insert Table 2//

3.4 Correlations between acylethanolamides and congeners and severity of depression and cognitive-affective and somatic symptoms

The severity of depression was positively correlated with DGLEA ($r_s = 0.304$; $p < 0.05$), OEA ($r_s = 0.249$; $p < 0.05$) and POEA ($r_s = 0.287$; $p < 0.05$). Only DGLEA ($r_s = 0.267$; $p < 0.05$) and POEA ($r_s = 0.258$; $p < 0.05$) correlated positively with the cognitive-affective symptoms. However, 2-LG ($r_s = 0.290$; $p < 0.05$), 2-OG ($r_s = 0.238$; $p < 0.05$), AEA ($r_s = 0.350$; $p < 0.01$), LEA ($r_s = 0.247$; $p < 0.05$), DEA ($r_s = 0.298$; $p < 0.05$), DGLEA ($r_s = 0.306$; $p < 0.05$), DHEA ($r_s = 0.281$; $p < 0.05$) and OEA ($r_s = 0.320$; $p < 0.01$) correlated positively with somatic symptoms (See Table 2).

//Insert Table 3//

//Insert Figure 3//

3.5 Multivariate discriminative model between depressive and control groups and between mild and moderate depression groups

All acylethanolamides, endocannabinoids and congeners were included as predictors in a multivariate logistic regression model to discriminate between the depressed patients group and the control group. A backward stepwise method was used to select the variables with the highest discriminative values. After 8 iterations, a model with 2-AG, 2-OG, and OEA as predictors showed a

good calibration using the Hosmer-Lemeshow test (Chi-square test = 6.270; $p = 0.617$). The ROC curve for this model showed an AUC = 0.847 ($p < 0.001$). The representative cut-off value was 0.5602 (sensitivity = 76.81; specificity = 78.72). The ROC curve and scatter dots for both groups are presented in Figure 3. The variables included in the final model are presented as supplementary material (See Table S9).

3.6 Differences in acylethanolamides and congeners between mild and moderate depression

The differences in endocannabinoid and congener concentrations were also tested between patients with mild and moderate depression. Patients with moderate depression showed significantly higher levels of AEA ($U = 186.000$; $p < 0.05$), LEA ($U = 186.000$; $p < 0.05$), DGLEA ($U = 156.000$; $p < 0.01$), and POEA ($U = 168.000$; $p < 0.01$) than those with mild depression (See Table 3).

//Insert Table 4//

3.7 Plasma concentrations of Oleoylethanolamide and 2-AG are elevated in depressed patients treated with SSRI

Patients using antidepressants showed higher levels of OEA ($U = 356.000$; $p < 0.05$), DGLEA ($U = 348.000$; $p < 0.05$) and 2-AG ($U = 295.000$; $p < 0.01$), than patients not receiving antidepressant therapy. Mann-Whitney U-tests found no statistically significant differences in the severity of depression between both samples (See Table 4).

4. Discussion

The present study reveals that depressed patients demanding treatment in primary care settings display alterations in their plasma concentrations of both, endocannabinoids and non-cannabinoid acylethanolamides, including 2-AG and OEA. A remarkable discovery is the association of the

elevations of the plasma concentrations of these two mediators in those patients under treatment with SSRI-type antidepressants. The antidepressant-induced rise of both 2-AG and OEA is relevant because these bioactive lipids display antidepressant activity in preclinical models of affective disorders (Khaturia et al., 2002; Jin et al., 2015). Moreover, there is an active clinical development of new antidepressant drugs based in the blockade of main endocannabinoid/acylethanolamide degrading enzyme fatty acid amidohydrolase – FAAH – with objective of rising the concentrations of these type of mediators (Bortolato et al. 2007). Whether SSRI-induced beneficial effects are derived, at least partially, from the elevations of these lipid mediators, remain to be conclusively determined. However, the positive association of these plasma concentration changes with the diagnosis of depression, and its link with SSRI therapy clearly supports the use of monitoring plasma concentrations of 2-AG and OEA as objective biomarkers relevant for clinical stratification of patients. Further research is needed to confirm this clinical use, although it is aligned with previous preclinical research findings.

Regarding 2-AG, we found a higher concentrations of 2- in patients than in controls, a finding that might be apparently controversial since main hypothesis is that this endogenous cannabinoid receptor agonist decreases along anxiety and depression (Hill et al., 2008), a finding supported by the initial observations of a negative affective state associated with cannabinoid CB1 receptor blockade (Navarro et al., 1997). For instance, the present data differs from those presented by Hill et al. (2008), who found lower concentrations of 2-AG in depressed women compared to controls, although the results of the AEA concentrations in depressed patients showed a similar tendency to those found in the present study. However we must have in consideration that the cohort of patients of the present study are not identified primary episodes without any previous intervention, as occurs with previous reports in humans suffering affective disorders (Hillard and Liu, 2014). Although we identify clearly this as a limitation of the study, the clear differences in cohort composition, therapy access and experimental conditions in previous clinical studies can account for the different profiles of plasma 2-AG concentrations reported. As an example, plasma 2-AG concentrations were found to be elevated in subjects completing a stress test (Hill et al. 2009). Finally, the increase in 2-AG concentrations reported here are basically found in patients under antidepressant therapy at the moment of recruitment, and this is the relevant finding. The rise on plasma 2-AG concentrations in SSRI-treated patients mirrors what has been described in the affective brain (Prefrontal cortex and amygdala) of animals treated with antidepressants, including SSRI (Smaga et al., 2014). Thus, we can state that the association of 2-AG and SSRI in

depressed patients is robust and aligned with main preclinical findings, supporting the involvement of this endocannabinoid in the pathogenesis and recovery from major depression.

Regarding non-cannabinoid acylethanolamides, especially OEA, the increase of its circulating levels in depressed patients has an antecedent in the study of Pavón et al. (2013) who described a similar rise of OEA and POEA in a sample of depressed cocaine users, in accordance with the present results. Our data suggest that apparently, the whole pathway generating acylethanolamides is activated in depression since all acylethanolamide concentrations were elevated in depressed patients. However, only OEA and DGLEA were further increased after antidepressant therapy. Interestingly, the correlational analyses between acylethanolamides and total severity, cognitive-affective and somatic depression scores showed that AEA, LEA, DEA, DGLEA, DHEA and OEA were correlated with somatic symptoms of depression and only DGLEA and POEA with cognitive-affective symptoms. These results underline the potential role of acylethanolamides, and in special OEA in the somatic symptoms of depression, such as crying, eating and sleeping disturbances and feeling fatigued. This hypothesis is supported by recent findings revealing that somatic symptoms of depression, as well as anhedonia, associated with LPS/ethanol injection are counteracted by OEA (Sayd et al., 2014; Anton et al., 2017). Since the analysis of the present data also supports for a role of 2-AG and related acylglycerols in somatic symptoms, it can be argued that endocannabinoids could be more related to these types of depressive symptoms than to the cognitive-affective ones. Preclinical evidence has revealed the influence of endocannabinoids and non-cannabinoid acylethanolamides in these type of behaviours (Rodríguez de Fonseca et al., 2001; Skaper and Di Marzo, 2012; Mechoulam and Parker, 2013).

Differences between patients with moderate and mild depression were also found in the present study. AEA, LEA, DGLEA and POEA were the molecules that better discriminated between mild and moderate depression. The differences found between patients with varying severities of depression indicated that subtle differences could be detected by analysing the plasma concentrations of endocannabinoids and congeners. Further studies with a larger sample would be needed to determine whether there is a differential pattern in the symptoms of mild and moderate depression. Considering the antidepressant actions of some acylethanolamides (Antón et al., 2017), this might indicate that overactivation of the ECS could be an endogenous antidepressant response, reducing the severity of depression symptoms. This hypothesis is aligned

with the development of inhibitors of acylethanolamide degradation for the treatment of depression, and we will have to wait until clear clinical evidence will be obtained in clinical trials with this type of new antidepressants.

Patients taking SSRI antidepressant medication showed higher levels of 2-AG, DGLA and OEA. There are no clinical studies that have investigated the relationship between these molecules and those involved in the SSRI antidepressant mechanisms. However, as we described in the introduction the pharmacological actions of OEA engages with serotonin, oxytocin and histamine transmission systems, that are all involved in the response to SSRI-type of antidepressants (Gaetani et al., 2010; Munari et al., 2015; Yu et al., 2015; Costa et al., 2018). Further research is needed to clarify whether this happens also in depressed human patients. Nonetheless, genetic studies have shown that some genes encoding FAAH (Monteleone et al., 2010) or a variant of the CNR1 gene could modulate responses to SSRI (Mitjans et al., 2012; Mitjans et al., 2013).

The results obtained are limited in varied aspects. First, the results of the analyses in which samples are divided by sex, severity of depression and medication are limited by the size of the samples. However, in all cases, the subsequent samples are balanced. Specific studies of these variables or a study with a larger sample size is needed to confirm the validity of these results. Second, we lack information about the chronology of the changes. Therefore, causal conclusions are not plausible. Third, given that OEA has been described to act as a satiety factor, potential changes in body weight during the course of the disorder might have altered its levels and, ideally, BMI should have been controlled in two time points. However, correlation analyses showed that BMI is not correlated with OEA levels (see Tables S7 and S8). Finally, although there are reports that prove that there are parallel changes in both peripheral and central concentrations of endocannabinoids (Plaza-Zabala et al., 2010), peripheral plasma concentrations cannot be directly related to central plasma concentrations. Therefore, caution is needed when considering conclusions about the role of the central nervous system in the presence of these acylethanolamides in depression. However, in support of this hypothesis, recent studies have shown that experimental treatments with OEA delivered peripherally can alleviate experimental depression in rodents (Anton et al., 2017).

The results of this study could also support the realization of translational research involving basic models of depression, pharmacological research, or even genetic studies on the different components of the endocannabinoid/acylethanolamide signalling systems to gain a

better understanding of the mechanisms by which these transmitters participate in major affective disorders.

ACCEPTED MANUSCRIPT

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Conflicts of interest

The authors have no conflict of interest to disclose.

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Legend to Figures

Figure 1. Differences (Means and SE) between controls, and depressed patients with and without antidepressant medication in the concentration of monoacylglycerols and acylethanolamides (Student t-test). * $p < 0.05$, two-tailed

Figure 2. Differences (Means and SE) between between controls, and depressed patients with and without antidepressant medication in the concentration of acylethanolamides (Student t-test). ** $p < 0.01$, two-tailed

Figure 3. ROC analysis and Scatter dots for discriminative model between depressed patients and controls using 2-AG, 2-OG, and OEA as predictors. AUC, Area Under the Curve; SE, Standard Error.

Table 1. Sociodemographic data.

Variable		p-value	Control group n = 47		Depressive group n = 69	
Age (yrs) [mean(SD)]		0.065	39.55	(11.55)	43.23	(9.64)
Sex [n (%)]	Men	0.683**	16	(34)	20	(29)
	Women		31	(66)	49	(71)
Marital status [n (%)]	Single	0.004**	20	(42.6)	9	(13)
	Married/Couple		23	(48.9)	49	(71)
	Divorced/Separated		3	(6.4)	9	(13)
	Widow		1	(2.1)	2	(2.9)
Education [n (%)]	Primary	0.015**	2	(4.3)	10	(14.5)
	Secondary		18	(38.3)	37	(53.6)
	University		27	(57.4)	22	(31.9)
Employment [n (%)]	Employed	0.000**	41	(87.2)	18	(26.1)
	Unemployed		3	(6.4)	46	(66.7)
	Student		3	(6.4)	5	(7.2)
BMI(kg/m ²) [mean (SD)]		0.882*	25.27	(4.42)	25.53	(5)
BDI (rank: 0-63) [mean (SD)]			-		24.54	(7.68)
Pharmacological Treatment	Antiinflammatories	-			1	(1.4)
	Antihypertensives	-			4	(5.8)
	Antidepressants	-			22	(31.9)
	Anxiolytics	-			24	(34.8)

BMI: Body mass index; BDI: Beck Depression Inventory; * p-value from Student's t-test or Mann-Whitney's U test; ** p value from Fisher's exact test or chi-square test

Table 2. Correlations between acylethanolamides and total BDI scores, cognitive-affective BDI scores and somatic BDI scores in depressed patients (n=69)

Concentration (ng/ml)	<i>Total BDI</i>	<i>Cognitive-affective BDI</i>	<i>Somatic BDI</i>
	r_s	r_s	r_s
OEA	0.249*	0.181	0.320**
POEA	0.287*	0.258*	0.211
LEA	0.197	0.165	0.247*
DGLEA	0.304*	0.267*	0.306*
DEA	0.234	0.184	0.298*
DHEA	0.138	0.046	0.281*
AEA	0.187	0.118	0.350**

r_s , Spearman's Rho. * $p < 0.05$, two-tailed; ** $p < 0.01$, two-tailed

Table 3. Differences in acylethanolamides between groups (with SSRI antidepressants vs without SSRI antidepressants)

Concentration (ng/ml)	Without antidepressants Mean (SD) n=47	With antidepressants Mean (SD) n=22	t	P	g
OEA	3.073 (0.713)	3.622 (1.115)	-2.475*	0.016	0.638
POEA	0.092 (0.042)	0.114 (0.068)	-1.640	0.106	0.426
LEA	1.353 (0.382)	1.355 (0.277)	-.021	0.983	0.006
DGLEA	0.083 (0.018)	0.100 (0.034)	-2.789**	0.007	0.703
DEA	0.145 (0.029)	0.143 (0.040)	.321	0.749	0.061
DHEA	0.507 (0.169)	0.544 (0.163)	-.841	0.403	0.221
AEA	0.540 (0.123)	0.583 (0.137)	-1.318	0.192	0.337

SD, Standard deviation; t, Student's t; g, Hedge's g (effect size); * $p < 0.05$, two-tailed; ** $p < 0.01$, two-tailed

Table 4. Differences in monoacylglycerols between groups (with SSRI antidepressants vs without SSRI antidepressants)

Concentration (ng/ml)	Without antidepressants Mean (SD) n=47	With antidepressants Mean (SD) n=22	t	P	g
2-AG	6.144 (5.588)	10.338 (5.590)	-2.432*	0.018	0.750
2-LG	129.298 (181.005)	112.163 (128.777)	0.399	0.691	0.103
2-OG	65.469 (83.899)	76.067 (78.743)	-0.498	0.620	0.093

SD, Standard deviation; t, Student's t; g, Hedge's g (effect size); * $p < 0.05$, two-tailed

Figure 1. Differences (Means and SE) between controls, and depressed patients with and without antidepressant medication in the concentration of monoacylglycerols and acylethanolamides (Student t-test). * $p < 0.05$, two-tailed

Figure 2. Differences (Means and SE) between controls, and depressed patients with and without antidepressant medication in the concentration of acylethanolamides (Student t-test). ** $p < 0.01$, two-tailed

Figure 3. ROC analysis and Scatter dots for discriminative model between depressed patients and controls using 2-AG, 2-OG, and OEA as predictors. AUC, Area Under the Curve; SE, Standard Error.

HIGHLIGHTS

- Oleoylethanolamide (OEA) is a non cannabinoid acylethanolamide with antidepressant activity in preclinical models.
- Depressed patients recruited at a primary care setting were found to have increased plasma levels of oleoylethanolamide and 2-arachidonoylglycerol.
- These finding was observed only in depressed patients with current antidepressant treatment with selective serotonin reuptake inhibitors (SSRI).
- The concentrations of OEA correlated with somatic symptoms of depression
- A potential role for OEA in the pharmacological actions of SSRI is discussed.