Reduced urine pregnenolone concentration after clinical response in patients with depression: an open-label prospective longitudinal study

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Abstract

Background

Identifying biological alterations in patients with depression, particularly those that differ between responders and non-responders, is of interest to clinical practice. Biomarker candidates involve neurosteroids, including pregnenolone (PREG) and allopregnanolone (ALLO). However, alterations in neurosteroids associated with treatment response are understudied. This study's main aim was to evaluate the effects of antidepressant treatment, clinical response, and treatment duration on neurosteroids PREG and ALLO in depression.

Materials and Methods

In a 4-week, open-label trial, participants were allocated randomly to the venlafaxine (n = 27) or mirtazapine (n = 30) group. Urine concentrations of PREG and ALLO were assessed through gas chromatography-mass spectrometry. Participants collected night urine between 10:30 p.m. and 8:00 a.m. The primary outcome was the effect of treatment (mirtazapine or venlafaxine), clinical response (operationalized through the Hamilton Depression Rating Scale), and time (baseline compared to 28 days) on the urine concentrations of PREG or ALLO in depression. Secondary outcomes were the effect of clinical response and time on the urine concentration of PREG or ALLO, independently of the antidepressant given (mirtazapine or venlafaxine). Linear mixed models were carried out.

Results

Regarding our primary outcome, there was no significant difference in PREG and ALLO concentrations between baseline and 28 days in responders and non-responders when investigating the venlafaxine or the mirtazapine group. However, concerning our secondary outcomes, we found a significant reduction of urine PREG concentration after 28 days of treatment in responders who received either venlafaxine or mirtazapine (estimate = -0.56; p = 0.016; 95CI [-1.003; -0.115]; Cohen's d = -0.61).

Conclusions

Our main results indicate that responders in depression show reduced urinary PREG concentrations after 4-weeks of therapy, independently of the antidepressant used. More studies are needed to confirm these findings.

Introduction

Among the most frequent mental health disorder, depression plays an important role by affecting almost 30% of the world population, according to the World Health Organization (1, 2). In addition, depression
represents high costs for health systems and is associated with high disability-adjusted life years.

Clinically, depression is characterized by anhedonia, loss of energy, diminished energy, depressed mood, excessive guilt, loss of concentration, hopelessness, and suicidal behavior (3, 4). Although there are many hypotheses concerning the etiology of depression, the exact causes and pathophysiology of depression are still unknown. However, various studies indicate possible biomarkers in depressive disorders, such as pro-inflammatory (e.g., IL-6 and TNF-α) (5, 6) and metabolic parameters (e.g., LDL/HDL cholesterol) (7–9).

Other biomarker candidates for depression reported in the literature are neurosteroids (10, 11), more specifically pregnenolone (PREG) and allopregnanolone (ALLO) (12, 13). PREG is synthesized by side-chain cleavage of cholesterol in mitochondria. The P450 side chain cleavage (P450SSC) gene is highly expressed in neurons and glia of the cerebral cortex and, to a lower extent, in the amygdala, hippocampus, and midbrain (14, 15). All other neurosteroids, including progesterone and ALLO, are derived from PREG. Furthermore, neurosteroids modulate the excitability of the brain via ligand-activated ion channels and influence general brain function, including emotion, motivation, and memory consolidation (16). In the case of depression, for instance, PREG and ALLO concentrations in cerebrospinal fluid have been reported to be lower in depressed patients compared to healthy controls (17, 18).

Interestingly, the disequilibrium of neurosteroids’ concentrations in depression is normalized after treatment, as shown in animal models (11). In a human study with depressive participants, levels of ALLO were increased in cerebrospinal fluid after treatment with selective serotonin reuptake inhibitors -SSRI- (i.e., fluoxetine and fluvoxamine), associating with Hamilton Rating Scale for Depression (HRDS) score improvement (18).

Since antidepressant agents could modify the concentration of neurosteroids, identifying variations of neurosteroids’ concentrations that discriminate between responders and non-responders to antidepressant treatment may be particularly interesting for clinical practice.

To date, the involvement of neurosteroids in the remission of clinical symptoms of depression is understudied and unknown. Moreover, it often remains unclear why some patients respond to the respective antidepressant while others do not. In concrete terms, identifying mediating biomarkers (such as neurosteroids) associated with treatment response in individuals could be of interest for individualized treatment of depression and lead to a more favorable course of the disease.

To this end, the following study has two main aims. Our first aim is to determine the effects of antidepressant treatment, clinical response, and treatment duration on the concentrations of PREG and ALLO in patients with depression. Our second aim is to determine if clinical response and treatment duration have significant effects on PREG and ALLO, independently of the antidepressant treatment used. Furthermore, we sought to determine the reduction pattern of depressive symptoms during
antidepressant treatment with mirtazapine or venlafaxine, two common antidepressants used in the
treatment of depression (19), with a potential effect on neurosteroids’ concentrations (20, 21).

Materials And Methods

Study design

The following study is a randomized, open-label prospective trial conducted at the Central Institute of
Mental Health (CIMH) in Mannheim (Germany). In this study, we compared two groups of patients with
two different antidepressant treatments: mirtazapine and venlafaxine. Both antidepressants have shown
in the literature an effect on neurosteroids’ concentrations (20-21), besides being the most prescribed
antidepressants in Germany after citalopram (19). In addition, we measured urine concentrations of two
neurosteroids, PREG and ALLO, to define whether there is a correlation between clinical response for a
specific treatment (mirtazapine vs. venlafaxine) and the reduction of both neurosteroids’ concentration.
During the open-label trial, participants continued to receive psychiatric inpatient treatments. The trial
consisted of two different time points: baseline and after 28 days of antidepressant treatment.

All participants were fully informed, and we received written informed consent before participation. This
study was conducted under the Helsinki Declaration and approved by the Ethics Committee of the
Medical Faculty Mannheim of the University of Heidelberg. The study is registered on the German Clinical
Trials Register (DRKS00000008) website.

Selection criteria and study participants

For this purpose, we recruited patients with depressive disorders from the inpatient unit for affective
disorders in the Central Institute of Mental Health (CIMH) of Mannheim.

As an inclusion criterion, participants must meet the DSM-IV-TR criteria for a depressive episode or major
depressive disorder (MDD), evaluated through semi-standardized clinical interviews (SCID-I) by two
experienced psychiatrists (MG, BS). Moreover, all participants must have > 18 years old, have a Hamilton
Depression Rating Scale (HDRS) score of at least 18 points before starting the open trial, and sign the
informed consent to be included in this trial. Finally, participants were included in the study if data
registration of the variables age, sex, BMI, PREG, and ALLO at baseline was completed.

General exclusion criteria for this open trial were the withdrawal of participation through informed
consent, underage participants, and HDRS < 18 points. Other medical exclusion criteria for this study were
the presence of anemia, hepatic or renal failure, a body-mass index (BMI) < 16 kg/m², and the use of
steroids at the moment of the study (22). Furthermore, participants who regularly took statins, lipid-
lowering agents, and/or oral antidiabetic agents were excluded from the study. In addition, participants
who changed habitual nutrition (e.g., following a diet to lose weight) were also
excluded (9,23). Concerning possible psychiatric diagnoses as exclusion criteria, participants with concomitant substance dependence, bipolar disorders, or schizophrenia-spectrum disorders (e.g., paranoid schizophrenia, schizoaffective disorders) were also excluded from the study. Finally, participants with a conservatorship or legal protectors, patients who could not give their consent because of severe mental illness, and participants with an acute exacerbation or life-threatening worsening of the mental status (e.g., suicide attempts, acute psychosis) were excluded from the study.

Initially, we recruited 105 participants with a depressive episode or MDD. However, two were excluded for not meeting the inclusion criteria, and eleven revoked their informed consent. The remaining 92 patients were randomly assigned in approximately equal numbers (1:1) to two groups: a venlafaxine group (VG) and a mirtazapine group (MG). However, we excluded patients due to acute life-threatening worsening of depression during treatment (2 participants of MG), drug-serious adverse events (3 participants of VG), withdrawal of informed consent during the treatment period (5 participants of VG, and 1 participant of MG) and premature discharge from the CIMH (1 participant of VG, and 1 participant of MG). Due to incomplete data, mainly concerning neurosteroids (PREG and ALLO), we excluded 10 participants of VG and 12 of MG.

Finally, 57 participants ($n_{VG} = 27; n_{MG} = 30$) remained, completed the open trial, and were included in the analysis.

**Trial course**

Before starting the open trial, participants underwent a one-week washout, which consisted of suspending any psychotropic drug (including antidepressants). The purpose of this period was to reach a drug therapy-naivety and to avoid any interaction effects. However, it was only allowed to use lorazepam or zolpidem *pro-re-nata* (PRN) for restlessness or insomnia, respectively (23).

During this washout period, patients were routinely evaluated by two experienced psychiatrists (MGi, BSc), assessing psychopathological examinations and HDRS for depressive symptoms (baseline evaluation). If patients showed HDRS scores < 18 points by the end of the washout period, they were excluded from the study, considering them as “responders during washout”.

After six days of the washout period, participants were allocated randomly to two groups, as mentioned above, and were treated either with mirtazapine or venlafaxine over 28 days. In case of mirtazapine, this was given once at 9:30 p.m. On the other hand, venlafaxine was given either once at 8:30 a.m. or on two occasions, at 8:30 a.m. and 12:30 p.m. Concerning drug doses, both antidepressants were administered at a minimum dose of 30 mg for mirtazapine and 75 mg for venlafaxine, with dose flexibility otherwise (23).
During the treatment periods, the course of the disease was documented weekly using the 21-item-HDRS. The evaluations were made on the 7th (first evaluation), the 14th (second evaluation), the 21st (third evaluation), and the end of the study (28th day, last evaluation). With the HDRS scores, we defined “response” as a ≥ 50% score reduction at the end of the study compared to baseline and “remission” as HDRS ≤ 7 at the end of the study (23).

Finally, to assess the neurosteroids ALLO and PREG, night urine was collected after the wash-out period (baseline) and on the last trial day (28th day, after psychotropic drug therapy) from 10:30 p.m. to 8:30 a.m. of the next day. After collection, we aliquoted and stored the urine samples at -30°C in separate boxes, which we later sent to a research facility (Institute of Endocrinology, Prague, Czech Republic) for analysis.

Assessment of neurosteroids

Gas chromatography-mass spectrometry (GC/MS) was used to analyze the concentrations of ALLO and PREG (24). In brief, free steroids were extracted from urine by diethyl-ether; steroid conjugates were hydrolyzed and extracted. The resulting residues were derivatized by methoxyamine hydrochloride and analyzed by GC/MS. Steroids were purchased from Steraloids (Newport, RI, USA), Sylon B from Supelco (Bellefonte, PA, USA), methoxylamine hydrochloride from Sigma (St. Louis, MO, USA), and solvents from Merck (Darmstadt, Germany).

Steroid levels were measured on a GCMS-QP2010 Plus system by Shimadzu (Kyoto, Japan) consisting of a gas chromatograph equipped with automatic flow control, an AOC-20s autosampler, and a single quadrupole detector with an adjustable electron (voltage of 10-195 V). A capillary column with a medium polarity RESTEK Rxi column (diameter 0.25 mm, length 15 m, film thickness 0.1 μm) was used for analyses. Electron impact ionization with electron voltage fixed at 70 V and emission current set to 160 μA was used. The injection port, ion source, and interface temperatures were maintained at 220 °C, 300 °C, and 310 °C, respectively. Analyses were carried out in the splitless mode with a constant linear velocity of the carrier gas (He), which was maintained at 60 cm/s. The septum purge flow was set at 3 ml/min. The samples were injected using the high-pressure mode (200 kPa), which was maintained for 1 min. The detector voltage was set to 1.4 kV.

Outcomes

The primary outcome of this open-label trial was the effect of treatment (mirtazapine or venlafaxine), clinical response (operationalized through HDRS), and treatment duration (28 days) on the urine concentrations of PREG or ALLO of participants from an inpatient unit with depression. Additionally, our
secondary outcome was to determine the effect of clinical response and treatment duration on the urine concentration of PREG or ALLO, independently of the antidepressant given (mirtazapine or venlafaxine).

Finally, we included variations in the HDRS scores through the trial period in both treatment groups. In this case, as suggested elsewhere, we defined clinical response if the HDRS total values were reduced to ≥ 50% and remission as a final score of ≤ 7 points (23,25,26).

Statistical analysis

Numeric variables that fitted a Gaussian distribution are specified in the text as mean (standard deviation). Numeric variables with a non-Gaussian distribution (i.e., median ≠ mean) are shown as median (interquartile range, 3rd quantile – 1st quantile). Categorical variables and count data are specified as numbers or fractions. Data with more than two decimals were rounded. Values smaller than 0.005 are presented as < 0.005 and values greater than 1 million are expressed in scientific notation (6). Descriptive data in the text are presented in tables. To evaluate the significance of differences between groups, t-tests were used for continuous normally distributed data and the U-test for continuous non-Gaussian data. We used the χ² or the exact Fisher test for the categorical and count data. For these differences, p-values were calculated. This study defined statistical significance whenever the two-tail-p-value was less than or equal to 0.05.

Primary and secondary outcomes were analyzed using general linear mixed models (LMM). Firstly, we calculated the primary outcome’s interaction time (i.e., baseline, after 28 days) * response (i.e., response, no response) * treatment (mirtazapine, venlafaxine). After that, we calculated time * response for the secondary outcome in another LMM and removed the variable “treatment” from the model to make it independent of the antidepressant treatment. The interactions were corrected for gender, age, and BMI confounding factors. In addition, we log-transformed the concentrations of PREG and ALLO using natural logarithms. We carried out multiple imputations with linear interactions and a maximum of 1000 iterations in case of missing values (five missing values). For both groups, PREG and ALLO levels were estimated in multiple imputations using the variables gender, BMI, and age of the participants. In the LMM, fixed effect omnibus tests were carried out to define the main effects of the variables in the model and to evaluate the variable in the model against the null model. The results of the LMM were reported graphically, and results are described in the text using p-values (significance threshold: p = 0.05) and 95% confidence intervals (95CI). When needed, post-hoc tests were carried out for the LMM when differences between the time * response * treatment and the time * response were significant.

For the statistical analyses (especially for LMM) and the descriptive data, we used the R-based software jamovi 2.0.0 (27) and the toolbox GAMLj (28). Finally, we generated the graphs using Prism 8 GraphPad (GraphPad Software Inc., California, United States of America).

Results
General characteristics of the sample

Table 1 shows the general characteristics of the sample, including HRDS scores, PREG, and ALLO concentrations in urine. Concerning HRDS scores, we found a significant reduction in the total HRDS score over time, with a decrease of 12.50 points in 28 days (p < 0.001; 95CI [-10.85; -14.14]). Moreover, responders to treatment showed a significant reduction of the HRDS score over time (Figure 1A; p < 0.001; 95CI [-4.45; -11.03]). In addition, both therapy groups (venlafaxine and mirtazapine groups) showed a significant reduction of HRDS scores within 28 days (Figure 1B; VG: Md = 15.87, t = 18.23, df = 52, p < 0.001; MG: Md = 16.94, t = 17.22, df = 52, p < 0.001). However, there were no significant differences between both groups (venlafaxine and mirtazapine group) regarding baseline HRDS values (t = 1.46, df = 85.13, p = 0.846) and after 28 days (t = 0.90, df = 85.13, p = 1.000).

Additionally, no differences were found between both groups concerning BMI (t = 1.32, df = 55, p = 0.192), age of participants (t = 0.85, df = 55, p = 0.399), and sex (p = 0.589).

<table>
<thead>
<tr>
<th></th>
<th>Venlafaxine group (VG; n = 27)</th>
<th>Mirtazapine group (MG; n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of participants</td>
<td>50.89 (14.89)</td>
<td>47.33 (16.46)</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>10/17</td>
<td>9/21</td>
</tr>
<tr>
<td>BMI (in kg/m^2)</td>
<td>26.93 (6.22)</td>
<td>24.86 (5.64)</td>
</tr>
<tr>
<td>Dosage (in mg)</td>
<td>213.89 (57.74)</td>
<td>46.00 (8.75)</td>
</tr>
<tr>
<td>HRDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>23.11 (4.04)</td>
<td>22.50 (4.23)</td>
</tr>
<tr>
<td>7 days</td>
<td>15.04 (6.27)</td>
<td>13.57 (6.27)</td>
</tr>
<tr>
<td>14 days</td>
<td>12.59 (5.87)</td>
<td>12.50 (6.08)</td>
</tr>
<tr>
<td>21 days</td>
<td>6.93 (5.22)</td>
<td>10.58 (6.37)</td>
</tr>
<tr>
<td>28 days</td>
<td>8.15 (5.69)</td>
<td>9.41 (6.49)</td>
</tr>
<tr>
<td>Percentage of responders (%)</td>
<td>85.19%</td>
<td>62.06%</td>
</tr>
<tr>
<td>PREG*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.31 (0.58)</td>
<td>7.42 (0.50)</td>
</tr>
<tr>
<td>28 days</td>
<td>7.18 (0.68)</td>
<td>7.44 (0.58)</td>
</tr>
<tr>
<td>ALLO*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.82 (1.00)</td>
<td>4.80 (0.97)</td>
</tr>
<tr>
<td>28 days</td>
<td>4.88 (0.91)</td>
<td>4.74 (0.83)</td>
</tr>
</tbody>
</table>

Table 1 - General characteristics of the sample. Quantitative data is described as mean (standard deviation). Raw values of PREG and ALLO are expressed in pmol. * Values were log-transformed with natural logarithms. Abbreviations: HRDS = Hamilton Rating Scale for Depression, BMI = Body-mass index, m = male participant, f = female participant, PREG = Pregnenolone, ALLO = Allopregnanolone.

Primary outcome – reduction of neurosteroids after antidepressant therapy (mirtazapine or venlafaxine) between clinical responders and non-responders
When investigating the interaction *time* *response* *treatment* with PREG concentrations, the results of the linear mixed model revealed no significant difference in PREG concentrations between baseline and 28 days in responders and non-responders when investigating the venlafaxine or the mirtazapine group (estimate = -0.10; p = 0.817; 95CI [-0.52; 1.12]; *Cohen’s d* = -0.06) (Figure 2A). Interestingly, independent of the *therapy group* (venlafaxine and mirtazapine) and *time* (baseline and 28 days), PREG concentrations associated significantly negatively with HRDS scores (estimate = -0.03; p = 0.041; 95CI [-0.06; -0.002]; *Cohen’s d* = -0.43).

Similar to the PREG results, we found no significant difference in ALLO concentrations between baseline and 28 days in responders and non-responders when investigating the venlafaxine or the mirtazapine group.

When investigating the interaction *time* *response* *treatment* with ALLO concentrations, no significant differences in ALLO concentrations between baseline and 28 days in responders and non-responders when investigating the venlafaxine or the mirtazapine group was found (estimate = -0.197; p = 0.787; 95CI [-1.62; 1.22]; *Cohen’s d* = -0.08) (Figure 2B). However, we found a significant negative association of ALLO with the *age of participant* (estimate = -0.019; p = 0.010; 95CI [-0.03; -0.01]; *Cohen’s d* = -0.78).

**Secondary outcome – reduction of neurosteroids and clinical response independently from antidepressant therapy (mirtazapine or venlafaxine)**

Concerning our secondary outcome, the results of the linear mixed model revealed a significant interaction *time* *response* with PREG concentrations (estimate = -0.56; p = 0.016; 95CI [-1.003; -0.115]; *Cohen’s d* = -0.61) (Figure 3A). Hereupon, a post hoc analysis was carried out, obtaining significant differences in the responders concerning PREG concentrations between baseline and 28 days (Figure 1; *M_D* = 0.68; p = 0.047). No significant differences between these two time points for the non-responders were found (Figure 3A).

Finally, we found only a significant association of ALLO with the *age of participant* (estimate = -0.018; p = 0.016; 95CI [-0.032; -0.002]; *Cohen’s d* = -0.70), similar to the results presented above. However, our results showed no significant interaction *time* *response* for ALLO (estimate = 0.084; p = 0.827; 95CI [-0.667; 0.835]; *Cohen’s d* = 0.05) (Figure 3B).

**Discussion**

We investigated the effects of antidepressant treatment (mirtazapine or venlafaxine), clinical response, and time (baseline versus 4 weeks of treatment) on the neurosteroids PREG and ALLO urinary concentrations in patients with depression. As our secondary outcome, the effect of clinical response and time on the urine concentration of PREG or ALLO was investigated independently of the antidepressant given (mirtazapine or venlafaxine). Our results showed no significant effects of antidepressant treatment (i.e., venlafaxine or mirtazapine), clinical response, and time on PREG or ALLO concentrations in people with depression. However, clinical response and time significantly affected PREG concentrations,
irrespective of the antidepressant used, when investigating patients who received either venlafaxine or mirtazapine. In this case, clinical responders showed significantly reduced urinary PREG concentrations over the treatment duration. There were no effects of clinical response or time on urinary ALLO concentrations in participants with depression, independently of the antidepressant given. Both mirtazapine and venlafaxine groups showed a significant reduction in depressive symptoms. Yet, when both treatments are compared, no significant differences regarding depression symptom reduction were found.

To the best of our knowledge, this is the first study determining a reduction of urinary concentrations of PREG in a group of clinical responders following antidepressant treatment. However, this effect was independent of the antidepressant treatment. Although there are no studies involving urinary concentrations of neurosteroids in therapy response and type of antidepressant in depression, similar studies analyzed serum PREG and other serum neurosteroids. For instance, a longitudinal study with major depressive disorder inpatients in treatment with fluoxetine showed no changes during the treatment of serum PREG after clinical remission achieved with fluoxetine (29). Likewise, an ECT study found no significant differences concerning serum PREG concentrations between baseline and clinical remission after treatment in people with treatment-resistant depression (30). However, both studies were performed with a small number of patients and considered only serum PREG, restricting the extrapolation of the results to our findings. In our study, neurosteroids were measured in the urine, which allows for estimating the excretion of these neuromodulators. Conjugated steroids are measured, which do not necessarily associate directly with neurosteroid concentrations in blood or tissues.

In addition, different neurosteroids may be altered differentially upon depression and treatment response. The relatively short half-life of PREG must be considered, whereas its metabolites, such as PREG sulfate (PREG-S), have a longer half-life and show a different effect on the hypothalamic-pituitary axis (31).

Finally, it is well known that inhibiting the hypothalamic-pituitary-adrenal axis (HPAA) system could occur under antidepressant therapy (32–35). This depends mainly on the choice of the antidepressant (e.g., mirtazapine = tricyclic antidepressants > SSRI = serotonin-norepinephrine reuptake inhibitor) and, to a lesser extent, on the therapy response (32, 35). Moreover, the adrenocorticotropic hormone (ACTH), an important component of the HPAA system, stimulates the conversion of cholesterol to PREG (36). This process is not brain-specific and is likely to occur primarily in the adrenal cortex. In this context, one interpretation of our results could be those antidepressants, if successful in inducing a therapeutic response, could inhibit the HPAA system and, thus, PREG synthesis. However, further studies are needed to confirm this.

In the current study, we have found a reduction in urinary concentrations of PREG during treatment in patients with depression who were clinical responders. However, previous reports showed decreased levels of PREG in cerebrospinal fluid or serum in people with depression before starting a treatment, which could be a possible pharmacological approach (37–40). We found that clinical response to depression is associated with a lower urinary elimination of PREG. This may suggest that clinical
response is associated with a higher concentration of PREG in the central nervous system, which may
have a neuroprotective effect (15). Nevertheless, more longitudinal studies are needed to confirm this
relationship between urinary PREG concentrations, PREG concentrations in cerebrospinal fluid, and
clinical response after treatment of depression over time.

In addition to our main results, we found a significant association between ALLO with the participant's
age. Different studies have already demonstrated the decline of neurosteroids in aging (41). In addition,
the enzyme 5α-reductase, which catalyzes the reaction of progesterone to 5α-DHP, could explain the age-
specific difference in ALLO excretion since its mRNA expression or sensitivity decreases with age (14).

Regarding our secondary aim, there were no significant differences in depression symptom reduction in
the individual treatment groups with mirtazapine and venlafaxine. This differs from a systematic review
and meta-analysis that reported mirtazapine to have a significantly higher antidepressant effect than
venlafaxine within two weeks of treatment, including higher remission rates (42). On the other hand, other
studies have reported that both antidepressants show a significant improvement in depressive symptoms
and remission and that there were no significant differences concerning the clinical response to
venlafaxine or mirtazapine (43). We have found a response rate of 85.19% for the venlafaxine group and
62.06% for the mirtazapine group, differing from a study that has seen a higher response rate of 52% for
the venlafaxine group compared to a response rate of 62% in the mirtazapine group, although not
statistically significant (44).

Although this study is the first to report urine concentrations of the neurosteroids PREG and ALLO
concerning antidepressant therapy, clinical response, and treatment duration, some limitations should be
considered. Firstly, the trial duration was 4 weeks (28 days). Therefore, no conclusions can be drawn
about long-term results, considering that antidepressant effects are sometimes not observed until more
than 4 weeks after the start of treatment. Secondly, urinary samples represented nighttime urine and were
collected only twice within the 4-week treatment (i.e., at baseline and after 28 days). Although this
allowed for assessment of the differences in neurosteroid excretion before and after four weeks of drug
administration, any cyclical or non-linear fluctuations within this range were not recorded. Cyclic
fluctuations may be relevant for ALLO, which is lower in women suffering from premenstrual syndrome
on the 26th cycle day than in healthy controls on the same cycle day (45), or in women with a
premenstrual syndrome who show reduced ALLO concentrations compared to controls in the luteal phase
(46). Thirdly, antidepressant effects would be better assessed in a placebo-controlled trial with a larger
sample size. Finally, the neurosteroid excretions in the urine were measured. This does not allow for direct
conclusions on effects in the brain, as concentrations are not necessarily directly proportional to each
other. However, neurosteroids can easily cross the blood-brain barrier due to their lipophilic nature. A
positive correlation between the serum concentrations (and excretion in the urine depends on this) and
the cerebrospinal fluid concentrations in the brain can be assumed. This positive correlation has already
been shown in a comparative study between cerebrospinal fluid and plasma concentrations of
neurosteroids (47). However, as recommended before, more studies are needed to investigate the
relationship between cerebrospinal fluid concentrations of neurosteroids, their urine elimination, and
clinical response in depression to observe how treatment and response modify the neurosteroids’ concentrations.

In light of our results, we conclude that PREG concentrations in urine are lower in patients that respond to pharmacological treatment after four weeks of treatment, independent of the antidepressant used. Concerning the used antidepressant (e.g., venlafaxine or mirtazapine), no differences were observed in the clinical response over time and concerning symptom reduction, indicating similar clinical effectiveness of venlafaxine and mirtazapine. Our results suggest that patients who respond to antidepressant therapy show reduced urinary PREG concentrations after 28 days of antidepressant therapy, while patients who do not respond to antidepressant therapy show unaltered PREG concentrations after 28 days of antidepressant treatment. In the long term, identifying biological alterations in patients who respond to antidepressant therapy could allow for individualized and, thus, more effective antidepressant treatment. However, future studies are needed to confirm this finding, comparing the reduction of urinary PREG concentrations over time in patients treated with other antidepressant classes (e.g., SSRI or NMDA antagonists).

References

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**Declarations**

The authors declare no conflicts of interests.

**Figures**
Figure 1

Estimated marginal means (with 95% confidence intervals) of Hamilton Depression Rating Scale (HDRS) scores and its variations between (A, left) treatment responders vs. non-responders and (B, right) patients with venlafaxine vs. mirtazapine.
Figure 2

Estimated marginal means (with 95% confidence intervals) of (A, left) urinary PREG concentrations and (B, right) urinary ALLO concentrations. The interaction time * response * treatment for urinary PREG concentrations (p = 0.817) and urinary ALLO concentrations (p = 0.787) was not significant. PREG and ALLO concentrations were log-transformed (natural logarithms) before being entered into the analysis.
Figure 3

Estimated marginal means (with 95% confidence intervals) of (A, left) urinary PREG concentrations and (B, right) urinary ALLO concentrations. The interaction time * response was presented. Here, we did find significant results for the interaction time * response for urinary PREG concentrations (p = 0.016) but not for urinary ALLO concentrations (p = 0.827). PREG and ALLO concentrations were log-transformed (natural logarithms), before entered into the analysis.