White matter volume and treatment with selective progesterone receptor modulator in patients with premenstrual dysphoric disorder

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ABSTRACT
Premenstrual dysphoric disorder (PMDD) is a mood disorder for which selective progesterone receptor modulator (SPRM) treatment has been demonstrated to be beneficial. The neural signatures of this treatment have been so far identified as greater fronto-cingulate reactivity during aggressive response to provocation, but no changes in terms of gray matter structure. White matter has recently been found to differ between patients with PMDD and healthy controls. The present study thus sought to investigate the relationship between white matter volume and SPRM treatment in patients with PMDD. A pharmaco-neuroimaging study was conducted on patients with PMDD participating in a randomized controlled trial. Participants underwent magnetic resonance imaging before and after treatment randomization to ulipristal acetate (an SPRM), or placebo, for three months. The interaction effect of treatment by time on white matter volume (WMV) was assessed. Voxel based morphometry analyses were performed on both a whole brain exploratory level and on regions of interest. No treatment effect was observed on WMV in any region, including the anterior thalamic radiations, cingulum, forceps minor, fornix, inferior fronto-occipital fasciculus, superior cerebellar peduncle, superior longitudinal fasciculus, and uncinate fasciculus. This is the first finding to indicate that no white matter volume alterations follow three-month progesterone antagonism, suggesting that white matter volume does not participate in symptom relief upon SPRM treatment for PMDD.

1. Introduction
Premenstrual dysphoric disorder (PMDD) is a menstrual cycle-related mood disorder that entails symptoms of depressive mood, irritability, anxiety, and mood lability, occurring during the luteal phase of ovulatory menstrual cycles and ceasing upon menstrual bleeding (Epperson et al., 2012). Luteal-phase symptomatology is temporally associated with progesterone fluctuations. Progesterone is highly lipophilic and can readily cross the blood brain barrier, while progesterone receptors are available in different brain regions that partake in affective processing, such as the frontal cortex, amygdala, hippocampus, hypothalamus (Brinton et al., 2008; Guerra-Araiza et al., 2000). Recently, a potential new treatment, based on a selective progesterone receptor modulator (SPRM), i.e. ulipristal acetate, showed promising results in treating the core emotional symptoms of PMDD, namely depression and irritability (Comasco et al., 2021). SPRMs comprise synthetic steroids that structurally mimic progesterone and, depending on the coactivator/pressor availability, and therefore on the progesterone receptor, they can alter the conformation which would then lead to agonistic or antagonistic effects. Upon binding on progesterone receptors, in a tissue-dependent manner, SPRMs affect transcription processes. After SPRM administration, low and stable progesterone levels, accompanied by mid-follicular estradiol levels are expected, along with amenorrhea in the majority of users (Rabe et al., 2018; Whitaker et al., 2014). Additionally, SPRMs are documented to inhibit ovulation by interacting directly with the pituitary gland and thus hindering or
reducing luteinizing and follicle stimulating hormone release (Rabe et al., 2018). To identify neural correlates that may explain the beneficial effects of the SPRM ulipristal acetate in patients with PMDD would contribute to understand the neural underpinnings of the symptomatology.

Menstrual cycle phase- and ovarian hormone-dependent effect on white matter have been demonstrated (Barth et al., 2016; De Bondt et al., 2013; Meeker et al., 2020). Specifically, higher mean diffusivity (MD) in the fornix has been found in monophasic combined oral contraceptive (COC) users compared with regularly cycling individuals in the follicular phase. Further, fornix MD was negatively correlated with estradiol and luteinizing hormone levels in both COC and non-COC users (De Bondt et al., 2013). Barth and colleagues revealed fractional anisotropy in the hippocampus to be negatively associated with estradiol levels (Barth et al., 2016). More recently, parietal white matter volume changes across the menstrual cycle were observed, while also positively correlated with estradiol levels (Meeker et al., 2020). Even though there is no direct evidence demonstrating a specific impact of progesterone on brain morphology, preclinical research points to the hormone’s effects on neuroplasticity (Camacho-Arroyo et al., 2020).

White matter is associated with myelin processes and alterations in white matter integrity and structure are relevant to psychiatric disorders (Fields, 2008). In particular, white matter changes have been observed in treatment-responsive individuals suffering from bipolar disorder or depression in key regions of emotion and reward circuits (Cattarussi et al., 2022; Espanhol and Vieira-Coelho, 2022; Tateishi et al., 2019). The neural structural signatures of PMDD have just begun to be clarified by studies on gray and white matter metrics (Dubol et al., 2020; Dubol et al., 2022; Gu et al., 2022). Regarding white matter, recent findings indicate larger uncinate fasciculus white matter volume in patients with PMDD compared with healthy controls (Gu et al., 2022).

While frontal-cingulate activation during aggressive response to provocation has been found to be higher in patients taking SPRM compared to placebo (Kaltsoni et al., 2021), three month SPRM treatment does not alter gray matter volume and cortical surface structure (Kaltsoni et al., 2022). Considering the known neuroplasticity effects of progesterone (Brinton et al., 2008; Camacho-Arroyo et al., 2020; Sakamoto et al., 2002), the recently observed menstrual cycle effects on white matter volume (Meeker et al., 2020), and the preliminary differences between individuals with PMDD and healthy controls in white matter volume (Gu et al., 2022), we aimed to further investigate the structural correlates of progesterone antagonism. Here, we assessed if SPRM treatment yielded structural alterations over time, by examining if white matter volume (WMV) would be a relevant neural correlate of SPRM treatment in patients with PMDD. Due to the limited knowledge about the relationship between WMV, PMDD, and progesterone antagonism, no directional hypothesis was posed.

2. Material and methods

2.1. Sample and study design

The sample was part of a double-blind randomized placebo-controlled trial and consisted of 35 participants, of which 18 were randomized to active treatment with SPRM and 17 to placebo for three treatment cycles (Kaltsoni et al., 2022). This study’s CONSORT diagram is presented in (Kaltsoni et al., 2022). All study procedures were approved by the ethics committee of Uppsala (Dnr. 2016/184) and the Medical Products Agency in Sweden, EUDRA-CT 2016-001719-19. Prospective symptom recording over two months was implemented to confirm the PMDD diagnosis according to DSM-5 criteria, using the Daily Report Severity of Problems (DRSP) scale (Endcott et al., 2006). A 50% increase from the follicular to luteal phase in at least five out of eleven symptoms is required for diagnosis, while at least one out of five symptoms must be one of the core PMDD symptoms (depressive symptoms, affective lability, irritability/anger, anxiety) (American Psychiatric Association and Association, 2013). As illustrated in Fig. 1, participants underwent two structural magnetic resonance imaging (MRI) sessions. The first scan took place during the luteal phase before randomization (baseline), at the end of this session subjects were randomized to either low-dose (5 mg per day) ulipristal acetate (Esmya® provided by Gedeon Richter Nordics AB) or identical-looking placebo tablets. Participants underwent the second scan toward the end of the 3-month treatment period (follow-up) (Comaschi et al., 2021).

Menstrual cycle phase was confirmed through estradiol and progesterone blood serum concentrations. Liquid chromatography—tandem mass spectrometry was used to measure serum hormonal concentrations at the Core Facility of Metabolomics, University of Bergen. Sample’s data were processed through robotization and protein precipitation was conducted with acetonitrile and liquid—liquid extraction with ethylacetate–heptane. A Waters Acuity UPLC system connected to a Waters Xevo TQ-S tandem mass spectrometer was used to analyze the samples. Compound separation was done on a C-18 column (50 × 2.1 mm, 1.7 mm particle size), which is developed by gradient elution over 14 min, using water and methanol containing ammonium hydroxide as mobile phases. They were detected in negative (e.g. oestradiol and oestrone) or positive ion (progesterone and testosterone) MRM mode. Two product ions are monitored for each compound to check for interferences. The method is highly selective and separates several isomers, e.g. epitestosterone and testosterone. The method is validated for oestradiol (sensitivity 3.6 pmol/L (LLQ) and 1.2 pmol/L (LOD) and total control volume (CV) for intermediate concentrations 5.0%), progesterone (10.3%), testosterone (3.2%), and cortisol (1.9%). Conjugated and non-conjugated steroids were analysed in separate runs with slightly different gradient.

2.2. MRI acquisition

A 3.0 T whole-body scanner was used (Achieva dStream, Philips Medical Systems). A 32-channel head coil was used to acquire 220 transversal slices using a magnetization-prepared rapid gradient echo sequence using the following parameters: repetition time 8.3 ms; echo time 3.8 ms; voxel size 0.94 × 0.94 × 1 mm³; matrix 256 × 256; flip angle 8°; 220 transversal slices; acquisition time 3 min, 50 s.

2.3. Data preprocessing and statistical analysis

All structural images were preprocessed using SPM12 (Wellcome Trust Center for Neuroimaging). Images were reoriented to the anterior commissure (set as origin: 0, 0, 0), spatially normalized and modulated to Montreal Neurological Institute (MNI) space, intensity variation corrected and segmented into tissue probability maps (Ashburner and Friston, 2000), using the SPM12 segmentation routine. Segmented WM probability maps were smoothed 8 mm full-width at half maximum Gaussian kernel, with final voxel size of 1.5 × 1.5 × 1.5 mm³.

Treatment effect on white matter volume was assessed using a voxel-wise non-parametric, mixed-effects model that assessed the timepoint (baseline vs. follow-up) and treatment group (SPRM vs. placebo) interaction effect. Additionally, timepoint differences within each treatment group separately and treatment group differences in each time-point were assessed, while considering age, body mass index (BMI), and total intracranial volume (TIV) as nuisance covariates. The threshold-free cluster enhancement method was used, set at 5000 permutations and a pFWE < 0.05 threshold. In addition to the exploratory whole-brain approach, regions of interest (ROIs) where defined as previously described in (Gu et al., 2022). ROI masks were defined according to the HCP-842 atlas (Yeh et al., 2018) and included the following bilateral masks for the: anterior thalamic radiations, cingulum, forceps minor, fornix, inferior fronto-occipital fasciculus, superior cerebellar peduncle, superior longitudinal fasciculus, and uncinate fasciculus.
3. Results

3.1. Descriptives

The two groups did not differ in terms of either age, BMI, or symptom severity at baseline (for demographics, see (Kaltsouni et al., 2022)). In terms of hormone levels, estradiol, testosterone, and cortisol, did not differ between the two groups, neither before nor after treatment, while progesterone levels were lower for the SPRM group at follow-up, due to progesterone antagonism (SPRM: 3.3 nmol/L ±7.8; placebo: 18 nmol/L ±7.8; p = 0.01, d = -1.1). Total scores of DRSP (SPRM: 38.7 ±15.3; placebo: 57.7 ±19.6; p = 0.01, d = -1.1) and the core symptoms were lower in the SPRM group at follow-up; depressive mood (SPRM: 5.4 ±2.4; placebo: 7.7 ±3.4; p = 0.05, d = -0.8), anxiety (SPRM: 1.9 ±1.0; placebo: 2.7 ±1.1; p = 0.04, d = -0.8), and affective lability (SPRM: 3.8 ±2.1; placebo: 5.9 ±2.2; p = 0.01, d = -1.0) comparisons reached statistical significance, while irritability did not (SPRM: 4 ±2.0; placebo: 5.5 ±2.3p = 0.1, d = -0.7). The decrease in total DRSP score from baseline was significantly higher in the SPRM group. As for treatment response and remission, high treatment response was attained by the SPRM group (93.8%), computed as the summation of complete (56.3%) and partial remission (37.5%), compared with placebo (58.8%, 17.5%, and 41.2% respectively; χ2 = 5.5, p = 0.02).

3.2. Treatment group by time-point interaction effect on WMV

Non-parametric, permutation-based voxel-wise analyses were performed to investigate the effect of SPRM treatment over time, in comparison with placebo, on WMV. The interaction between time points and group, yielded no significant clusters, neither when assessing the defined ROI masks nor on a whole brain level (Fig. 2).

3.3. Treatment group time-point effects on WMV

WMV measured at baseline and follow-up was compared within each treatment group. No significant changes from baseline were found for either treatment group. Negative findings were the case for both whole-brain and ROI approaches.

3.4. Treatment group differences

The two treatment groups did not differ in WMV at follow-up. To exclude any pre-existing differences, we tested treatment group differences at baseline, observing no difference before randomization.

4. Discussion

The current findings point to the absence of WMV changes as a result of SPRM treatment in patients with PMDD. Additionally, no change over time was observed when the treatment and placebo group were assessed separately.

The current results, combined with the lack of an effect of SPRM treatment on gray matter structure (Kaltsouni et al., 2022), corroborate the hypothesis that structural alterations are possibly not part of the mechanism by which SPRM treatment leads to symptom relief in patients with PMDD, as demonstrated by the larger trial (Comasco et al., 2021). However, three months of administration can be regarded as a short time window in order for structural effects to be observed in white matter volume. Unfortunately, the literature on the neurobiological underpinnings of pharmacological interventions for PMDD remains limited (Sundström-Poromaa and Comasco, 2023) and the current results should therefore be replicated.

According to evidence based on rodents and lesion studies, progesterone’s impact on brain structure has been described mainly through neuroprotective effects on white matter (Guennoun, 2020). In a recent review on the effects of progesterone on the female brain, a positive effect of progesterone on myelination on female mice has been suggested (Pletzer et al., 2023), although highlighting the otherwise limited literature on females. In terms of ovarian hormones other than progesterone, estradiol concentrations have been positively correlated with hippocampal (Barth et al., 2016) and fornix white matter integrity (De Bondt et al., 2013). Due to this scarcity of investigations in animals and humans, it is not possible to draw direct inferences regarding the lack of structural effects of progesterone antagonism on the brain of...
patients with PMDD. Considering the positive effect of SPRM on PMDD mood symptoms (Comasco et al., 2021), it can be postulated that antagonizing progesterone seems to lead to functional changes (Kaltsouni et al., 2021), but that structural effects are not part of the mechanism behind symptom relief, as indicated by the lack of changes in gray matter morphology (Kaltsouni et al., 2022).

Conclusively, white matter macrostructure was not impacted by three-month treatment with SPRM in patients with PMDD. Further
research on the neural correlates of symptoms relief upon pharmacological interventions for PMDD is warranted to clarify the molecular mechanisms of both the disorder and symptom relief.

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**CRediT authorship contribution statement**

Kaltsouni Elavset: Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. Wikstrom Johan: Conceptualization, Data curation, Methodology, Project administration, Resources, Writing review & editing. Lanzenberger Rupert: Conceptualization, Methodology, Writing – review & editing. Sundstrom-Poromaa Inger: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. Comasco Erika: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

**Declaration of Competing Interest**

The study drugs were provided by Gedeon Richter, but they had no further involvement in the study design, data collection, analysis, findings’ interpretation, or manuscript preparation. RL received travel grants and/or conference speaker honoraria within the last three years from Bruker BioSpin MR, Heel, and support from Siemens Healthcare regarding clinical research using PET/MR. He is a shareholder of the start-up company BM Health GmbH since 2019. ISP has served occasionally on advisory boards or acted as invited speaker at scientific meetings for Asarina Pharma, Bayer Health Care, Gedeon Richter, Peptontics, Shire/Takeda, Sandoz, and Lundbeck A/S. All other authors declare that they have no conflict of interest.

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