



Treatment of premenstrual dysphoric disorder with the GABA_A receptor modulating steroid antagonist Sepranolone (UC1010)—A randomized controlled trial



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ABSTRACT

Context: Allopregnanolone is a metabolite from progesterone and a positive modulator of the GABA_A receptor. This endogenous steroid may induce negative mood in sensitive women when present in serum levels comparable to the premenstrual phase. Its endogenous isomer, isoallopregnanolone, has been shown to antagonize allopregnanolone effects in experimental animal and human models.

Objective: The objective was to test whether inhibition of allopregnanolone by treatment with the GABA_A modulating steroid antagonist (GAMSA) Sepranolone (UC1010) during the premenstrual phase could reduce symptoms of the premenstrual dysphoric disorder (PMDD). The pharmacokinetic parameters of UC1010 when given as a subcutaneous injection were measured in healthy women prior to the study in women with PMDD.

Design: This was an explorative randomized, double-blind, placebo-controlled study.

Setting: Swedish multicentre study with 10 centers.

Participants: Participants were 26 healthy women in a pharmacokinetic phase I study part, and 126 women with PMDD in a phase II study part. Diagnosis followed the criteria for PMDD in DSM-5 using Daily Record of Severity of Problems (DRSP) and Endicott's algorithm.

Intervention: Subjects were randomized to treatment with UC1010 (10 or 16 mg) subcutaneously every second day during the luteal phase or placebo during one menstrual cycle.

Outcome measures: The primary outcome measure was the sum of all 21 items in DRSP (Total DRSP score). Secondary outcomes were Negative mood score i.e. the ratings of the 4 key symptoms in PMDD (anger/irritability, depression, anxiety and lability) and impairment (impact on daily life).

Results: 26 healthy women completed the pharmacokinetic phase I study and the dosing in the following trial was adjusted according to the results. 106 of the 126 women completed the phase II study. Within this group, a significant treatment effect with UC1010 compared to placebo was obtained for the Total DRSP score ($p = 0.041$) and borderline significance ($p = 0.051$) for the sum of Negative mood score.

Nineteen participants however showed symptoms during the follicular phase that might be signs of an underlying other conditions, and 27 participants had not received the medication as intended during the symptomatic phase. Hence, to secure that the significant result described above was not due to chance, a post hoc sub-group analysis was performed, including only women with pure PMDD who completed

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the trial as intended ($n=60$). In this group UC1010 reduced Total DRSP scores by 75% compared with 47% following placebo; the effect size 0.7 ($p=0.006$), and for sum of Negative mood score ($p=0.003$) and impairment ($p=0.010$) with the effect size 0.6. No severe adverse events were reported during the treatment and safety parameters (vital signs and blood chemistry) remained normal during the study.

Conclusions: This explorative study indicates promising results for UC1010 as a potential treatment for PMDD. The effect size was comparable to that of SSRIs and drospirenone containing oral contraceptives. UC1010 was well tolerated and deemed safe.

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1. Introduction

Premenstrual dysphoric disorder (PMDD) affects 3–5% of women in fertile age (Sveindottir and Backstrom, 2000; Wittchen et al., 2002). The disorder is typified by a recurrent cluster of mental symptoms such as irritability, depressed mood, aggression and emotional lability that consistently recur only in the premenstrual (luteal) phase of the menstrual cycle (APA, 2013; O'Brien et al., 2011). Quality of life for these women is reduced due to a significant negative impact on social life, relations and work performance during the premenstrual period (Dennerstein et al., 2010). The pathophysiology of PMDD is not yet fully understood, but a temporal association with circulating ovarian steroids, in particular progesterone and its metabolite allopregnanolone (3 α -OH-5 α -pregnan-20-one), has been established (Backstrom et al., 2003). There are several lines of evidence suggesting the involvement of progesterone/allopregnanolone in PMDD. Most importantly, symptoms are relieved (or even abolished) when ovarian hormones are suppressed (Wyatt et al., 2004), and are reinstated when progesterone is administered (Segebladh et al., 2009). With the use of functional magnetic resonance imaging (fMRI), several studies report changes in brain reactivity across the menstrual cycle, most notably increased amygdala reactivity in the luteal phase (Toffoletto et al., 2014). Furthermore, throughout the brain, the highest concentration of progesterone is found in the amygdala (Bixo et al., 1997). The effect, however, is probably not induced by progesterone itself since the classical progesterone receptor antagonist, mifepristone (RU486), does not ameliorate the symptoms (Chan et al., 1994). Further, increasing evidence suggest that the symptoms are mediated by a progesterone metabolite, allopregnanolone, normally active as a positive modulator on the GABA(γ -amino-butyric acid)_A receptor. Inhibition of progesterone conversion to allopregnanolone has been shown to ameliorate the symptoms in PMDD women (Martinez et al., 2016), and symptoms are strongly correlated to a specific level of allopregnanolone (Andreen et al., 2009).

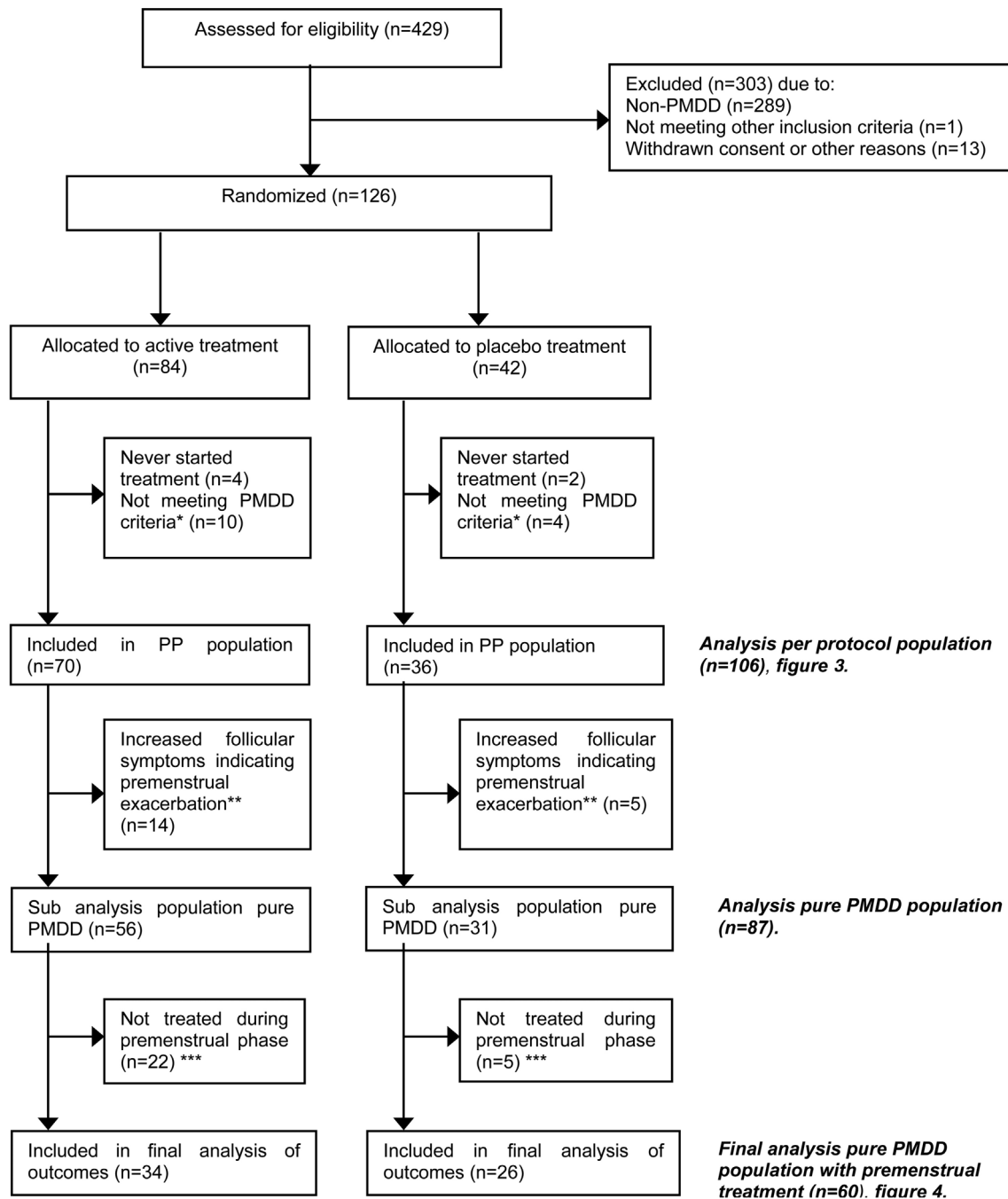
Allopregnanolone is normally a potent positive GABA_A receptor modulating steroid (Bristot et al., 2014) and like other positive GABA_A receptor modulators, such as benzodiazepines and barbiturates, it has, in high concentrations, anaesthetic, antiepileptic and anxiolytic properties in animals and humans (Timby et al., 2006; van Broekhoven et al., 2007). Given its rapid conversion, serum levels of allopregnanolone mirror those of circulating progesterone across the menstrual cycle (Bixo et al., 1997; Wang et al., 1996). However, simple relationships (such as an excess or deficiency of allopregnanolone in women with PMDD) have not been established in systematic studies (Backstrom et al., 2003). Nevertheless, in women with PMDD the premenstrual mood improves when serum levels of allopregnanolone decrease (Martinez et al., 2016; Nyberg et al., 2007). Concentrations of allopregnanolone, corresponding to normal luteal phase levels, induce more severe mood changes than both higher and lower levels indicating a bimodal effect of allopregnanolone on mood (Andreen et al., 2006; Hommer et al., 1986). In line with these results, an abnormal response to physio-

logical serum levels of ovarian steroids in women with PMDD was also shown by Schmidt et al. (Schmidt et al., 1998). In addition, fMRI studies have revealed a similar paradoxical response since a low oral dosage of progesterone, producing low serum concentration of allopregnanolone, increases amygdala reactivity, whereas a high dose decreases amygdala reactivity during an emotion discrimination paradigm (van Wingen et al., 2007; van Wingen et al., 2011). Similar bimodal/paradoxical effects are well described for other GABA_A receptor agonists, e.g. benzodiazepines, in a subgroup of the general population (Bramness et al., 2006; Dougherty et al., 1996; Wenzel et al., 2002).

One likely reason for the altered response to allopregnanolone in PMDD is the plasticity of the GABA_A receptor, since subunit composition and pharmacological properties has been shown to change with different reproductive states (Lovick et al., 2005; Maguire et al., 2005). For example, progesterone treatment or concentrations of progesterone/allopregnanolone across the estrous cycle, lead to an up-regulation of the $\alpha 4, \beta \delta$ receptor subunits in the hippocampus, which, in turn, render the receptor more sensitive to the effects of allopregnanolone (Belelli et al., 2002; Shen et al., 2005). Studies in mice show that allopregnanolone can increase anxiety in situations of increased $\alpha 4 \beta \delta$ GABA_A receptor expression in hippocampus. In these studies, allopregnanolone is probably exerting its action on $\alpha 4 \beta \delta$ containing GABA_A receptors because this effect was not seen in δ - or $\alpha 4$ -knock-out mice, and is probably acting as a negative modulator at $\alpha 4 \beta \delta$ containing receptors under certain conditions (Shen et al., 2007; Shen et al., 2013).

In experimental studies of healthy women, intravenous allopregnanolone dose-dependently increase sedation and decrease maximal saccadic eye velocity (SEV). Measurement of SEV can be used to quantify GABA_A receptor sensitivity (de Visser et al., 2003; Iacono and Lykken, 1981). In a recent study by us, women with PMDD were shown to have an altered sensitivity to an intravenous injection of allopregnanolone compared to healthy controls. PMDD women were more sensitive during the luteal phase of the menstrual cycle (Timby et al., 2016).

Allopregnanolone effects can be antagonized by its isomer isoallopregnanolone (Sepranolone; UC1010, 3 β -OH-5 α -pregnan-20-one) as shown in animal experiments (Backstrom et al., 2005; Lundgren et al., 2003; Shen et al., 2007; Stromberg et al., 2006), as well as in humans (Bengtsson et al., 2015). Isoallopregnanolone is a GABA_A modulating steroid antagonist (GAMSA) and does not antagonize the effect of GABA itself or other GABA_A agonists like benzodiazepines and barbiturates (Lundgren et al., 2003). When given intravenously to healthy women, isoallopregnanolone, does not cause any severe side-effects or adverse mood reactions as was shown in a pharmacokinetic study (Hedstrom et al., 2009). The hypothesis upon which the present study is based, is that the negative mood associated with PMDD is caused by the allopregnanolone-enhanced GABA-stimulated chloride uptake via primarily the GABA_A receptor in the emotional center of CNS, and that women with PMDD have an altered sensitivity to the increase in allopregnanolone concentration during the luteal phase. The treatment rationale for UC1010 (isoallopregnanolone) is thus based



*Criteria for analysis during treatment cycle: (1) received \geq four doses, (2) had $>$ 4 days of DRSP ratings during Day -6 to Day 1 and (3) showed ovulation on progesterone test (n=14 did not fulfil these criteria).

**Nineteen patients showed follicular phase symptoms above the 97.5 % confidence limit of the total population, see figure 3.

***27 patients were not exposed to treatment during the premenstrual, symptomatic period.

Fig. 1. The flow of patients through the study part 2.

on its ability to modulate recombinant human $\alpha 1, \beta 2, \gamma 2L$ GABA_A receptor function (Rahman et al., 2008), through a different mechanism than pregnanolone sulphate (Wang et al., 2007). This effect could very well be receptor sub-type specific as demonstrated in the $\alpha 4 \beta 2 \delta$ GABA_A receptor subtype (Sabaliauskas et al., 2015).

So far, treatments focusing directly on PMDD are lacking and effects of different therapies are individual and varying.

Some women feel helped by birth control pills containing drospirenone (Lopez et al., 2012), others by selective serotonin reuptake inhibitors (SSRIs) (Marjoribanks et al., 2013). The only effective therapy includes induction of anovulation by Gonadotropin-Releasing Hormone (GnRH) agonists but this therapy is complicated and requires hormonal add-back (Segebladh et al., 2009).

The aim of the present study was to test whether repeated dosing of UC1010 subcutaneously in the luteal phase is superior to placebo in reducing mood symptoms in women with PMDD. Further aims were to assess drug exposure, safety and tolerability of UC1010 in the employed doses, preparation and regimen.

2. Materials and methods

2.1. Trial design

This was a randomized, double-blind, placebo-controlled parallel-group study on safety, tolerability, pharmacokinetics and pharmacodynamics of UC1010 administered subcutaneously, single-dosing in healthy women (part 1) and multiple dosing in PMDD women during one menstrual cycle (part 2) – an explorative phase I/II study. Moreover, it was a multi-center trial conducted at 10 study centers in Sweden (university or other tertiary hospitals, secondary hospitals and private clinics). Study part 1, which was a pharmacokinetic study of UC1010 to healthy female volunteers, was performed at two of the university hospital centers. The study was performed according to the Helsinki declaration and Good Clinical Practice. The study protocol and informed consent form were approved by the Regional Ethical Review Board in Stockholm (Dnr 2012/1715-31/3) and the Medical Product Agency of Sweden approved use of UC1010 in this study. The study is registered at www.clinicaltrials.org with identification no. NCT01875718 and EudraCT no. 2012-004081-18.

2.2. Participants

Subjects were recruited after advertisement in local newspapers and on the Internet. For study part 1, which was a pharmacokinetic study, 26 healthy women without PMDD were recruited.

Eligible women for study part 2 were initially pre-screened via a telephone interview conducted by trained study nurses, and thereafter screened with an electronic version of the DRSP (Daily Record of Severity of Problems), which is a validated instrument for PMDD diagnosis (Endicott et al., 2006), for two months prior to inclusion. A web-based version of the DRSP was used and PMDD was diagnosed following the criteria in DSM-5 by use of the algorithm described by Endicott et al. (Endicott et al., 2006). Inclusion criteria for both study parts were women of age 18–45, essentially healthy, regular menstrual cycles and non-hormonal contraception. Exclusion criteria were use of steroid hormones during three months prior to the study, use of psychotropic or anti-depressant drugs during one month prior to the study, significant physical or psychiatric conditions (apart from depression more than two years earlier), drug or alcohol abuse, pregnancy, regular night shift work or participation in another clinical trial. Subjects on SSRI treatment for PMDD were included after a one-month wash-out period. Physical examination, vital signs, pregnancy test, routine chemistry screens and M.I.N.I. (Mini-International Neuropsychiatric Interview) were performed at the screening visit to ensure that the participants were essentially healthy and not pregnant. All subjects provided written informed consent after oral and written information about the aim of the study and the study procedures.

2.3. Interventions

In study part 1, healthy women were randomized to two different doses of UC1010 (10 vs. 16 mg) or placebo. UC1010 was given as a single, subcutaneous injection, and thereafter blood samples were collected at timed intervals during 3 days. The results were used to determine the dosing of study part 2 according to the pharmacokinetic analysis with the aim to obtain a therapeutic target level of 2–10 nmol/L serum for at least 10–12 days of the luteal phase. The

pharmacokinetic analysis indicated that for the dosing in study part 2 an every-second day administration would suffice to reach the target exposure (see Results below).

In study part 2, participants received 5 subcutaneous injections of active drug (10 or 16 mg UC1010) or placebo during one menstrual cycle, starting at the time of ovulation (day after LH peak). Urine assays for measurements of LH were used (Clear Blue[®], SPD Swiss Precision Diagnostics GmbH, Geneva, Switzerland) by the subjects. However, the results were not controlled by the study personnel, a circumstance that was later identified as a weakness to the protocol. It turned out that the LH-test used to verify ovulation failed in some cases with the consequence that some patients were not treated as intended during the luteal phase.

UC1010 was suspended in an MCT (medium-chain triglyceride) oil-containing vehicle to a concentration of 25 or 40 mg/mL and manufactured on behalf of Asarina Pharma by Patheon, Swindon, UK. MCT only was used as placebo control. The dose volume was standardized to 0.4 ml. The exposures of study drug were anticipated to be essentially similar with both doses due to the characteristics of the drug product.

2.4. Outcomes

DRSP (Daily Record of Severity of Problems) is a validated instrument for PMDD diagnosis (Endicott et al., 2006), and has previously been used to measure treatment effects in PMDD patients (Cohen et al., 2002; Halbreich et al., 2002; Yonkers et al., 2005). The participants rate severity of 11 different PMDD-specific symptoms (explored by 21 questions) along with impact on social activities, relations and work performance on the DRSP in a Likert scale ranging from 1 to 6. When used for daily symptom screening during two months and with exclusion of differential diagnoses it adheres to the DSM-5 system for PMDD diagnosis. In study part 2, the pre-defined outcome variables were the sum of all 21 items (Total DRSP score) comprising a maximum score of 126 and minimum score of 21, the sum of scores for the four key symptoms (anger/irritability, depression, anxiety and lability; Negative mood score) with a maximum score of 48 and minimum of 8, and the impairment scores (effect on social activities, relations and work performance) with a maximum score of 18 and minimum of 3. The sum of scores for the best 5 consecutive days during day 5–12 in the menstrual cycle (follicular phase) was compared to the 5 worst consecutive days during day –6 to 1 (luteal phase) for establishing PMDD diagnose at baseline. The predetermined primary variable was the difference in symptoms from follicular phase (Fmin) to luteal phase symptoms (Lmax). Effect size was measured in relation to the respective patients' baseline score during the treatment cycle comparing the effect in the luteal phase between the groups.

2.5. Safety assessments

At baseline, medical history and concomitant medication were monitored. In addition, physical examination, vital signs, pregnancy test and safety lab tests (whole blood hematology and clinical chemistry screen) were performed. In study part 1, vital signs and inspection of the injection site were performed daily for 3 days after the injection. Safety lab tests were done on day 4 after the injection. Adverse events were recorded repeatedly for 2 weeks.

In study 2, vital signs and inspection of the injection sites were done at all 5 treatment visits. Physical examination and safety lab were repeated at the end of the treatment period. Adverse events were recorded continuously for 10 weeks.

2.6. Sample size

For study part 1, it was deemed that 20 (10 + 10) actively treated and 6 placebo treated women should suffice for a pharmacokinetic evaluation. Basis for a power calculation was lacking since this preparation of UC1010 has never been given subcutaneously to humans before.

For study part 2, a power analysis based on a simulated result on PMDD using the DRSP score indicated a need for 40 subjects per treatment group (including compensation for drop-outs). In this estimate, based on earlier treatment effects of other therapeutic agents (Cohen et al., 2002; Halbreich et al., 2002; Yonkers et al., 2005) it was assumed an improvement in placebo of 10 units compared to 16 units in the active group (both doses combined) with a common SD of 9.5 points. The study would then reach a power of 80% with alpha level 5%.

2.7. Randomization and blinding

The randomization was performed by an independent statistician, using the Excel random number generator. Subjects were randomized in equal blocks according to treatment arm for each study center. Packing and labelling of the test drug according to the randomization list was done by InPac Pharma, Lund, Sweden, prior to shipment to a central pharmacy for further distribution to the study sites. The study medication was filled in glass vials – the primary packing. Each vial was packed in one sealed carton, the secondary packing, and then in a larger box, tertiary packing, per subject, with 2 vials per subjects in study part 1 and 14 vials per subjects in study part 2. Since the active drug was different in appearance from the placebo solution, special measures were taken to hinder un-blinding, and the drug injections were given by independent un-blinded study staff not otherwise involved in the study procedures. The blinded study staff did not open the secondary packing of an individual subject prior to injection. Injection sites were the lower abdomen and the participants were not allowed to see the study medication. The un-blinded study staff sealed and signed the secondary packing and a separate monitor was responsible for drug accountability. Thus, investigators, other study staff, monitors and subjects were kept blinded to treatment allocation during the whole study.

2.8. Analysis of data and statistical methods

All data was analysed on a per-protocol (PP) basis. The PP population included in the outcome analyses was defined as all subjects i) having received at least 4 doses of test drug, ii) having at least 4 days of scorings on the DRSP during day –6 to day 1, and iii) having a verified ovulation during the treatment cycle. Missing values in the DRSP scoring were replaced using linear interpolation from the nearest available neighbouring value. For the effect variables, active treatment (predefined to be a composite of both UC1010 groups) was compared to the placebo group. The composite of the two UC1010 dosages was used since the exposures of study drug were anticipated to be similar due to the characteristics of the drug product.

The score value used in the calculations of the baseline and treatment cycles was the difference in the sum of all 21 symptoms (Total DRSP score) rated during the 5 worst luteal phase days (Lmax) minus the total DRSP scores during the 5 best follicular days (Fmin). The treatment effect on the Total DRSP score was then calculated as the difference in score during the treatment cycle compared to the total DRSP score during baseline (average of the two screening cycles, Fig. 2) intra-individually. The Kruskal Wallis and Mann-Whitney tests were used for group comparisons. The change in scorings of the 4 key symptoms (Negative mood score)

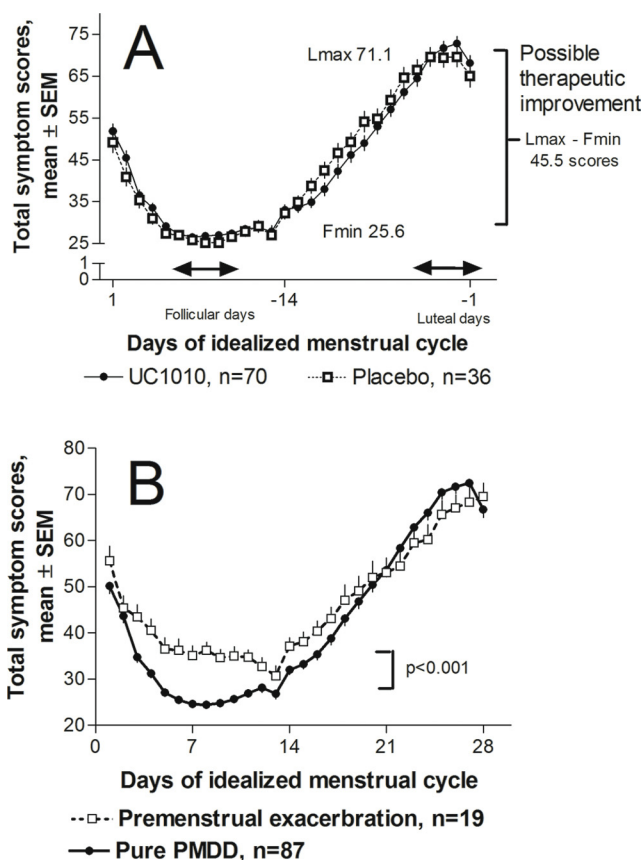


Fig. 2. Total DRSP score at baseline (minimal value = 21) men \pm SEM during 2 diagnostic cycles for (A) the group subsequently randomized to active treatment with UC1010 compared to the group randomized to placebo (n = 106) and (B) in subjects with pure PMDD and the subject with premenstrual exacerbation of symptoms.

and delta impairment scores, as well as individual symptoms, were analysed with the same non-parametric methods. Secondary sensitivity analyses, with covariates, were performed using step-wise forward models including both linear and logistic regression. All outcomes were tested 2-sided at 5% significance level. Effect size was calculated using Cohen's d approximation.

2.9. Subgroup analyses

During the work up of the data two major problems were revealed. Firstly, the M.I.N.I. was apparently not sensitive enough to detect all women with other underlying conditions and the diagnostic DRSP algorithm (Endicott et al., 2006) was apparently too permissive and did not efficiently exclude subjects with negative mood symptoms in the follicular phase. Secondly, the LH assays for urine testing indicated ovulation too early or too late, in some cases, with the consequence that 19 subjects were not treated as intended in the protocol during the symptomatic luteal phase and were not exposed to the test drug when the PMDD symptoms were present. To investigate that the obtained results in the PP-population were not due to random effects, a post hoc analysis was made to investigate what influence these study problems had on the primary analysis. Subjects included in these sub-analyses are displayed in Fig. 1.

To identify the patients with high follicular phase symptom scorings at baseline, the following statistical method was employed; an outlier was defined as a patient with at least one follicular phase symptom rated higher than the 97.5% confidence interval (CI) for that particular symptom in the total patient group studied.

3. Results

3.1. Subject characteristics

The study part 1 was performed between February and March 2013. Thirty-one women were screened and 26 were included in the study. They were essentially healthy with normal vital signs, and mean age 31.9 ± 7.0 years. Three women were not included due to eligibility reasons and two because the study quota was filled. Eight women were allocated to treatment with low dose UC1010, 11 women to treatment with high dose UC1010, and seven women to placebo. All 26 completed the pharmacokinetic study.

The study part 2 was performed between January 2013 and May 2014. In total, 429 women were screened, but only 140 of them fulfilled the criteria for PMDD during the screening period. The subject flow is shown in Fig. 1. Thirteen women were not included because the study quota was filled or their consent withdrawn and one (1) did not fulfill other inclusion criteria. 126 women were thus randomized but six did not start treatment and another 14 did not fulfill the predefined criteria for inclusion in the data analysis as described in the methods. Consequently, there were 106 patients included in the per protocol population (PP) on which the first statistical analysis was made.

Baseline scorings on the DRSP during the screening period did not differ between the groups later randomized to treatment with active drug vs. placebo (Fig. 2). Baseline characteristics were essentially similar (N.S.) in the treatment groups in study part 2 (Table 1).

3.2. Study drug plasma exposures

In study part 1 (pharmacokinetic study) the two doses of UC1010 were compared to placebo. Plasma levels in the treatment groups reached on average 4.2 nmol/L four hours after the injection with some inter-individual variation. Exposure to higher plasma concentrations than 2 nmol/L of UC1010 was established for 30 h in all subjects in the 10 mg dose group and for 40 h in six out of 11 subjects in the 16 mg dose group. The datasets of the 10 and 16 mg UC1010 dose groups were also carried through a compartment analysis. A one-compartment model with first-order input/output was fit to UC1010 plasma concentrations and the individual final parameter estimates were used for simulating repeated dose scenarios where a dose of either 10 or 16 mg was given every 24, 48 or 72 h five times. The results indicated that by dosing UC1010 every day, an accumulation would occur and thus plasma concentrations would exceed the therapeutic target interval. The dosing in study part 2 was therefore spaced out to every second day.

In the PMDD patients treated with UC1010, the drug exposures with the two doses given were not significantly different from each other. This was as anticipated and due to the characteristics of the drug product. On the day of the 4th or 5th dose, samples were taken before and approximately at 4, 6 and 8 h after the injection. The average highest plasma concentrations determined were 6.4 and 9.1 nmol/L in the 10 mg and the 16 mg dose groups, respectively, 4–6 h after injection.

3.3. Effects of UC1010 on PMDD symptom severity in the PP population

The predetermined primary variable was the difference in symptoms from follicular phase (Fmin) to luteal phase symptoms (Lmax). Fig. 2 shows the baseline ratings for all 106 subjects in the PP population (average of Total DRSP score from two diagnostic cycles). During treatment, a statistically significant larger improvement in Total DRSP score was obtained in the group that received UC1010 compared to placebo ($p=0.041$), Fig. 3, Table 2. The sum of four negative mood symptoms (Negative mood score)

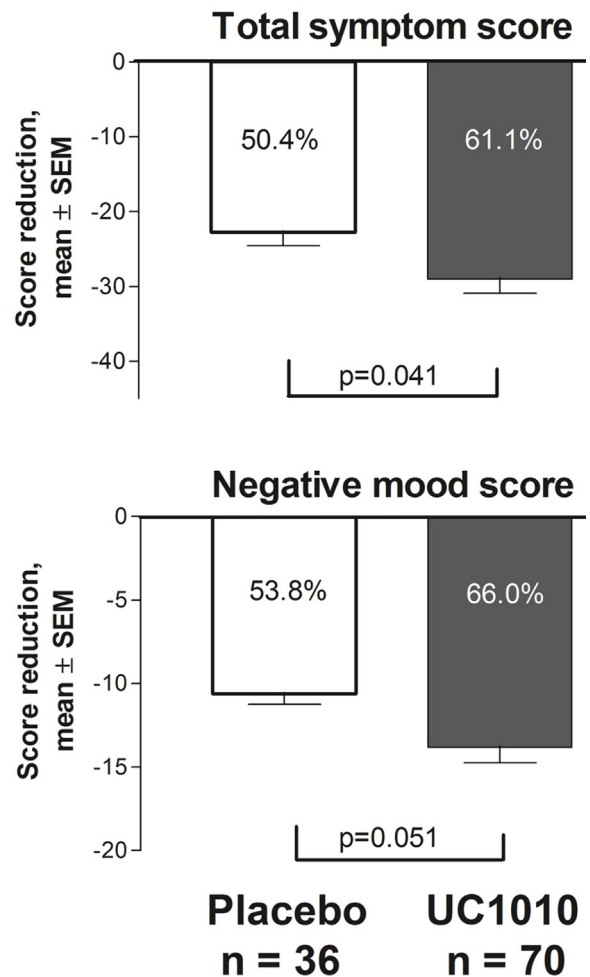


Fig. 3. Reduction of Total DRSP score (top panel) and Negative mood score (bottom panel) in the group treated with UC1010 compared to placebo (PP population, $n=106$). Mean reduction in ratings (\pm SEM) and % change from baseline are shown.

and impairment scores improved, but the analysis showed borderline significance ($p=0.051$ and 0.091 , respectively). The placebo effect was, as expected, approximately 50%. Among the individual symptoms “depression” and “out of control” improved significantly with treatment, but no statistically significant effects on the physical symptoms were detected (Table 2).

3.4. Subgroup analyses

A total of 19 patients were classified as having premenstrual exacerbation of continuously existing symptoms and were thus not essentially healthy. The remaining 87 subjects were free of symptoms during the follicular phase and regarded as having pure PMDD. The difference in baseline scorings was obvious in the group with pure PMDD compared to the group with premenstrual exacerbation as shown in Fig. 2. To examine if the statistically significant difference in treatment effect between UC1010 and placebo remained after the exclusion of patients with premenstrual exacerbation a sub-group analysis of the population with pure PMDD was done. In this group of 87 women, the treatment effect with UC1010 became stronger and the placebo effect somewhat less pronounced. The Total DRSP score improvements from baseline were 62.3% in the UC1010 treated vs. 48.1% for the placebo group ($p=0.016$), and differences for the mood symptom scorings (Negative mood score) and impairment were also larger ($p=0.009$ and $p=0.038$ respec-

Table 1
Baseline characteristics of subjects treated with UC1010 or placebo (PP population in study part 2, n = 106).

	UC1010, n = 70		Placebo, n = 36	
	Mean ± SD	Median (range)	Mean ± SD	Median (range)
Age, yrs	36 ± 6.0	37 (22–46)	36 ± 6.8	38 (19–46)
Parity, n	1.4 ± 1.1	2.0 (0–4)	1.3 ± 1.3	1.0 (0–4)
Menstrual cycle length, days	27 ± 2.0	27 (24–33)	28 ± 1.7	28 (25–31)
BMI, kg/m ²	23 ± 3.4	23 (17–36)	24 ± 3.3	23 (19–31)
Blood pressure systolic, mm Hg	116 ± 9.6	116 (95–142)	119 ± 12	119 (100–149)
Blood pressure diastolic, mm Hg	72 ± 6.4	71 (57–89)	73 ± 8.1	72 (60–87)
B-hemoglobin, g/L	132 ± 10.3	134 (105–153)	132 ± 7.5	133 (108–143)
S-creatinine, μmol/L	66 ± 8.4	66 (49–86)	68 ± 12	65 (51–110)
S-ASAT, μkat/L	0.37 ± 0.10	0.37 (0.19–0.77)	0.39 ± 0.14	0.34 (0.26–1.01)
S-ALAT, μkat/L	0.31 ± 0.11	0.29 (0.10–0.65)	0.37 ± 0.17	0.34 (0.13–0.92)

BMI = body mass index; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; SD = standard deviation. No statistical differences were seen between the two groups.

Table 2
Effects of UC1010 compared to placebo on symptoms of the premenstrual dysphoric disorder measured by Daily Rating of Severity of Problems (DRSP) in the PP population (n = 106) and in 60 women with pure PMDD treated during the premenstrual phase. The effects are calculated as the difference between luteal and follicular phase scores at baseline minus the corresponding difference during treatment.

	PP-population, n = 106			Pure PMDD population, n = 60		
	Placebo Mean ± SD n = 36	UC1010 Mean ± SD n = 70	P=	Placebo Mean ± SD n = 26	UC1010 Mean ± SD n = 34	P=
Total DRSP score ^a	22.8 ± 14.67	29 ± 19.94	0.041	21.5 ± 15.01	34.4 ± 21.26	0.006
Negative mood ^b	10.6 ± 6.83	13.8 ± 9.78	0.051	9.9 ± 6.24	16.8 ± 10.12	0.003
Impairment ^c	4.5 ± 3.11	5.3 ± 3.55	0.091	4.2 ± 2.65	6.1 ± 3.79	0.010
Depression	3.6 ± 2.87	5 ± 3.91	0.040	3.1 ± 2.63	6.1 ± 4.3	0.002
Anxiety	1.3 ± 0.84	1.8 ± 1.36	0.091	1.3 ± 0.83	2.2 ± 1.34	0.003
Lability	2.9 ± 2.23	3.6 ± 2.78	0.243	2.9 ± 1.96	4.4 ± 2.83	0.029
Anger/irritability	2.8 ± 1.84	3.4 ± 2.49	0.125	2.7 ± 1.65	4.1 ± 2.28	0.004
Usual activities	1.4 ± 1.21	1.7 ± 1.29	0.180	1.2 ± 1.01	2.1 ± 1.27	0.004
Concentration	1.2 ± 0.95	1.5 ± 1.32	0.266	1.1 ± 1	1.6 ± 1.45	0.121
Fatigue	1.2 ± 1.12	1.6 ± 1.35	0.084	1.2 ± 1.08	1.6 ± 1.27	0.102
Appetite	2.4 ± 2.43	2.1 ± 2.25	0.662	2.7 ± 2.39	2.5 ± 2.21	0.982
Sleep	1.8 ± 1.95	2.4 ± 2.36	0.142	1.6 ± 1.98	2.6 ± 2	0.039
Out of control	2 ± 2.13	3.1 ± 2.43	0.022	1.6 ± 2.13	3.5 ± 2.74	0.004
Physical	2.1 ± 2.93	2.8 ± 3.08	0.317	2.1 ± 3.29	3.5 ± 3.09	0.114

SD = standard deviation. P-values for group comparisons are based on Mann-Whitney *U* test followed by a step-wise forward model using logistic regression for correction of intra-individual follicular phase differences.

^a The sum of all 21 items in the DRSP.

^b Composite score of the four key symptoms depression, anxiety, lability and anger/irritability.

^c Negative impact on social activities, relations and work performance.

tively) than in the PP-population. Thus, a potential impact of the premenstrual exacerbation group on the overall results in the PP population was revealed.

As mentioned in the methods section above, the LH-test used to verify ovulation failed in some cases, and 27 patients of the 87 with pure PMDD had not been treated as intended during the luteal phase. In the group of subjects with pure PMDD who had been treated as intended in the protocol (n = 60), there was a statistically significant better treatment effect compared to placebo for Total DRSP score, $p < 0.006$, Negative mood score, $p < 0.003$, and summarized impairment score, $p < 0.01$, Fig. 4.

The therapeutically possible overall symptom improvement (Fig. 2) was 45.5 scale steps on the DRSP, and the actual treatment effect of UC1010 was –34.4. The effect constitutes a 75% reduction in symptoms. Thus, the effect size was 0.7 for Total DRSP scores ($p = 0.006$), and 0.6 for Negative mood score ($p = 0.003$) and impairment ($p = 0.010$).

Scorings of individual symptoms show that the treatment was effective in reducing mood symptoms, but not physical symptoms (Table 2). Age was not a significant covariate. Because of the variation in number of patients included at the 10 study sites, an ANCOVA was done to explore treatment by study center interaction, and this analysis revealed no significant effect.

3.5. Safety and follow-up

The 26 subjects in study part 1 and all 120 subjects entering the treatment cycle in study part 2 were evaluated for safety. No serious adverse events were reported during the treatment period in any of the study parts. Two participants withdrew from the study due to adverse events, AE (Fig. 1). The frequency of reported AEs was equal in the placebo and treatment groups in both study parts. 74 of the subjects in study part 2 reported an AE, and of those 8 were of moderate-severe severity and considered to possibly be related to the drug. AEs were equally reported in the active and placebo treated subjects and hence there were no safety concerns for UC1010 treatment. The most frequently occurring AE was related to the injection sites, including mild swelling and redness at the injection site in a few subjects and similarly frequent in the active and the placebo groups (more than 90% of injection sites were completely without reactions). Information about AEs was collected until 14 days after end of treatment. Between baseline and end of treatment period, only minor changes in the laboratory safety tests were noted but not considered clinically significant. Also, no significant changes in vital signs were seen and the lengths of the menstrual cycles were not affected by the treatment. 18 participants (10 placebo, 8 active drug) scored symptoms on the DRSP

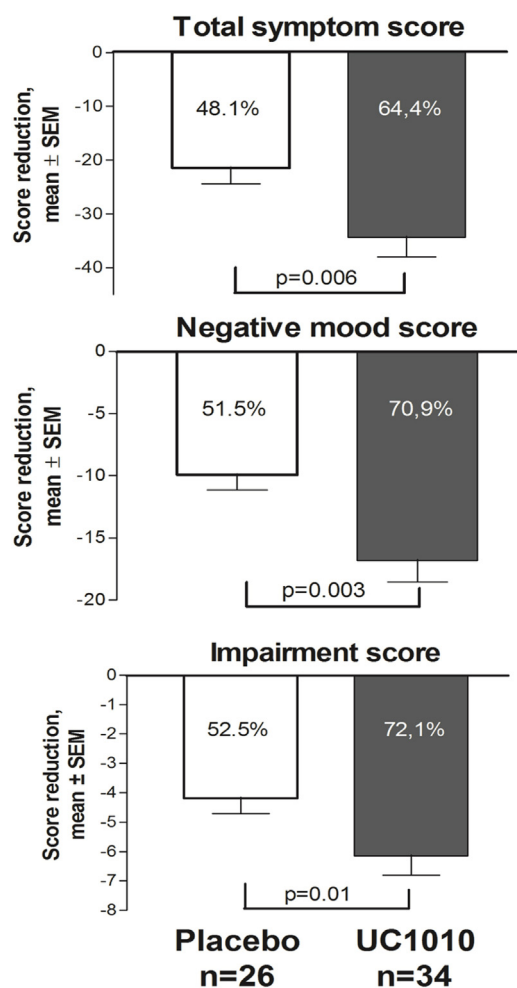


Fig. 4. Reduction of Total DRSP score (top panel), Negative mood score (middle panel) and impairment (bottom panel) in a sub-group with pure PMDD treated with UC1010 compared to placebo during the symptomatic luteal phase (n=60). Mean reduction in ratings (\pm SEM) and % change from baseline are shown.

during one follow-up cycle after the treatment and no significant changes compared to the screening cycles were noted.

4. Discussion

The study constitutes an explorative first-in-man treatment study using Sepranolone (UC1010) as luteal phase treatment for PMDD in a randomized, parallel-group, placebo-controlled study. The result show that UC1010 reduced PMDD symptoms significantly better than placebo, based on the per protocol analysis. The potential treatment effect for UC1010 is promising when administered more optimal. UC1010 was well tolerated and there were no clinically significant changes in safety variables. The rating scale used for assessment of treatment effects (DRSP), has earlier been used in studies approved by the Food and Drug Administration (FDA) in the U.S. where treatment effects of SSRIs (registration studies) and of drospirenone-containing oral contraceptives on PMDD were investigated. The summarized total symptom score was consistently used as primary measure in these earlier studies (Cohen et al., 2002; Yonkers et al., 2005). In the present study, the treatment effect in the PP population was statistically significant when the Total DRSP score was used as outcome and borderline significant when summarized negative mood (Negative mood score) was used.

Although the study is exploratory and results will have to be confirmed in a larger phase II trial, the results could be regarded as an initial “proof of concept”, strengthening the hypothesis that allopregnanolone is involved in the pathogenesis of PMDD and that UC1010, as a GABA_A modulating steroid antagonist (GAMSA), is a potential treatment for this condition. A proof of “target engagement” has earlier been reported for UC1010 in humans as UC1010 has been shown to antagonize allopregnanolone pharmacodynamics (Bengtsson et al., 2015).

Two post hoc analyses were done to confirm the per protocol results and to show the potential for a new treatment when given more optimal. The significance between UC1010 and placebo groups was not due to high presence of follicular phase symptoms as the difference between the UC1010 and placebo treatments remained after the women with premenstrual exacerbation of symptoms had been excluded from the analysis. However, the presence of follicular phase symptoms seemed to be of importance. The degree of reduction in symptoms by treatment with UC1010 was seemingly blunted if the patients suffer from a premenstrual exacerbation of an existing condition and not a pure PMDD. Moreover, in the present study the patients with high presence of follicular phase symptoms were unevenly distributed between groups in this relatively small study. In a future study the follicular phase symptoms have to be more strictly controlled than was done by the DRSP algorithm used in the present study. It is well known that some patients may have an undetected underlying condition even though it is not discovered by the psychiatric screening procedure at inclusion (Hammarback and Backstrom, 1989).

Timing of the treatment with UC1010 seems to be of importance. One third of the population in this study was not treated as intended during the premenstrual period due to technical problems with the ovulation test indicators. In most cases, the treatment was terminated too early because of this and it seems to be crucial that the women are exposed to UC1010 during the symptomatic period and until the start of menstruation. A reappearance of symptoms is likely to occur if the UC1010 exposure is interrupted prior to the decline in allopregnanolone serum levels. Nevertheless, the final subgroup analysis showed promising results again indicating that the original PP-analysis results are not due to a random effect. An analysis excluding only the women not treated as intended also showed significant treatment effects of UC1010 compared to placebo (data not shown). In the group of women with pure PMDD treated according to the protocol during the whole symptomatic luteal phase, consistent and statistically significant results were obtained. Regardless whether Total DRSP score, Negative mood score or impairment was measured, the treatment effect was statistically significant indicating a potential for treatment with UC1010 in PMDD. The therapeutically possible overall symptom improvement (Fig. 2), was -45.5 scale steps on the DRSP, and the actual treatment effect of UC1010 was -34.4 (-75%) and -21.5 for placebo (-47%). The effect constituting 75% reduction in symptoms could also be expressed as an overall shift from severe to mild/minimal on the DRSP scale. Thus, the effect size varied between 0.64–0.73 for Total DRSP score, Negative mood and impairment scores. This is similar to that seen in studies of SSRIs and drospirenone-containing oral contraceptives used as treatment for PMDD (Cohen et al., 2002; Halbreich et al., 2002; Yonkers et al., 2005). Notably, the treatment effect of UC1010 on negative mood symptoms was pronounced but the effect on e.g. physical symptoms was not statistically significant (Table 2), and obviously weakened the results when Total DRSP score was used as outcome in the analyses.

The aim of this study was to explore the pharmacokinetics and pharmacodynamics of the UC1010 preparation in a multi dosage trial and being a first-in-man study. In this explorative phase I/II study, only one treatment cycle was employed instead of three, which is recommended in a phase II and III trials (O'Brien et al.,

2011). The limited treatment period is a weakness to the study, especially since the placebo effect is usually greatest during a first treatment month as shown in earlier PMDD studies (Wang et al., 1995).

UC1010 was well tolerated and no safety concerns were identified. This is in contrast to treatments with SSRIs and oral contraceptives where side-effects are common and even some potential harms (e.g. thromboembolism) exist. Compliance with SSRI treatment is low and most patients end their treatment due to adverse effects (Sundstrom-Poromaa et al., 2000). UC1010 may thus have the potential to become a preferable alternative to the two treatments for PMDD available today. An intended future clinical therapy with UC1010 includes intermittent administration during the luteal phase. Pharmacokinetic data indicate that the 14-day wash-out period between treatment periods will be sufficient to completely restore basal levels of isoallopregnanolone. The results in the present study indicate that UC1010 in doses resulting in a slight elevation of the endogenous luteal phase concentration of isoallopregnanolone will be sufficient to achieve symptom alleviation. Also, it should be noted that this serum concentration of isoallopregnanolone is well within the physiological range seen during pregnancy. The better effect in the population treated as intended compared to those starting treatment too early, indicates that UC1010 should be present in appropriate levels during the late luteal phase to exert best possible effect.

In conclusion, the present result constitutes an initial indication that UC1010 reduces symptom severity and impairment significantly more efficiently than placebo especially in women with a well-defined, pure PMDD. If a larger clinical trial could confirm these findings, UC1010 (Sepranolone) constitutes a promising, well-tolerated treatment for PMDD.

Declaration of interest

Karin Ekberg was, at the time of the study, CEO of Asarina Pharma AB and Torbjörn Bäckström served as medical officer in the company and is also a shareholder.

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