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Raloxifene as a treatment for cognition in women with schizophrenia: the influence of menopause status

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ABSTRACT

Cognitive impairments cause significant functional issues for people with schizophrenia, often emerging before the onset of hallucinations, delusions and other psychosis symptoms. Current pharmacological treatments do not target cognitive dysfunction. Several lines of evidence support the beneficial effects of estrogens on cognition. Raloxifene hydrochloride, a selective estrogen receptor modulator, has been associated with cognitive improvements in healthy postmenopausal women and in schizophrenia, although findings are inconsistent. Using pooled data from two clinical trials, the aim of the current study was to compare the efficacy of 120 mg/day adjunctive raloxifene to placebo for 12 weeks on cognitive performance in women with schizophrenia who were stratified by menopause status (pre-menopausal; peri-menopausal or post-menopausal). A total of sixty-nine participants with a diagnosis of schizophrenia or schizoaffective disorder were included. Cognition was assessed at baseline and study end using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Results indicated that after stratifying for menopause status (strata) and adjusting for endogenous hormone levels (estrogen, progesterone, follicle stimulating hormone and luteinising hormone), semantic fluency, picture naming and list recognition change from baseline scores for the raloxifene group differed significantly from the placebo group. The findings from the current study highlight the importance of considering menopause status when interpreting the effects of hormonal treatments.

1. Introduction

Cognitive problems are a core feature of schizophrenia, with up to 80% patients showing significant impairment (Keefe and Fenton, 2007). Cognitive deficits are closely aligned with functional outcome and present a significant obstacle in the recovery process (Green, 1996). Current treatment options do not successfully ameliorate cognitive impairments (Green, 2006; Minzenberg and Carter, 2012). The areas of neurocognition that are primarily impaired include attention, working memory, memory as well as executive functioning skills, such as decision making, inhibition and planning (Minzenberg and Carter, 2012).

Sex hormones, particularly estrogen, have been substantially investigated over recent years in relation to their capacity to modify brain function. Findings from animal studies demonstrate that estrogens can influence spine density in the hippocampus and prefrontal brain regions (Tuscher et al., 2016; Woolley et al., 1990), promote neurotrophin synthesis (Milne et al., 2015) and protect the brain against stress and inflammation (Luine, 2016). Animal studies have demonstrated that estrogens have the capacity to enhance cognition, particularly in the areas of learning and memory (Engler-Chiurazzi et al., 2016; Luine, 2014). Human studies have also demonstrated beneficial effects of estrogen therapies, although findings are less consistent (Luine, 2014) and suggest that the effects of estrogens on cognition may be dependent on patient age, menopause status, duration of treatment as well as vary according to the cognitive domain assessed (Weickert et al., 2016).

The development of selective estrogen receptor modulators (SERMs), such as raloxifene, provide an estrogen therapy with mixed agonist/antagonist properties thus avoiding some of the adverse risks that have been associated with estradiol therapy (e.g. Beral et al., 2005). Raloxifene has antagonist effects on the estrogen receptor in the breast and uterus, while maintaining agonistic effects on the estrogen receptors in bone and brain tissue (Shang and Brown, 2002). Raloxifene, currently approved for use in postmenopausal women with osteoporosis, has been associated with mixed effects on cognition, potentially reflecting variations in methodology, dose and study populations (Yang et al., 2013).

A number of placebo-controlled studies in healthy postmenopausal women have shown little or no benefit of 60 mg/day raloxifene on

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cognitive function (Buckwalter et al., 2007; Haskell and Richardson, 2004; Jacobson and Truax, 1991; Nickelsen et al., 1999), with the exception of one study reporting verbal memory improvements in healthy late postmenopausal women (70–80 years) following 60 mg/day of raloxifene treatment at 3, 6, and 12 months (Jacobsen et al., 2010). A higher raloxifene dose of 120 mg/day (Nickelsen et al., 1999) has been associated with an improvement in verbal memory following 1 month of treatment (however this effect was no longer significant at 6 or 12 months). As part of the Multiple Outcomes of Raloxifene Evaluation trial, a large number of postmenopausal women with osteoporosis (n = 7478, mean age: 66) received either 60 mg raloxifene, 120 mg raloxifene or placebo for three years. The combined raloxifene group (60 mg and 120 mg groups combined) demonstrated a trend toward less decline following three years of raloxifene treatment on the two tests of verbal memory and attention (Yaffe et al., 2001). Further analyses revealed a reduced risk of developing mild cognitive impairment and a lower risk of developing Alzheimer's disease at the higher dose of 120 mg/day(Yaffe et al., 2005). A recent systematic review on the effects of raloxifene on cognition in postmenopausal women concluded that a dose of 120 mg/day may have some benefit for cognition in relation to ageing and risk of cognitive decline(Yang et al., 2013).

Clinical trials in schizophrenia have similarly revealed mixed findings. We have previously demonstrated a beneficial effect of 12 weeks of adjunctive raloxifene treatment (120 mg/day) on overall psychosis symptom severity, and positive psychosis symptoms in older women with refractory schizophrenia (Kulkarni et al., 2016, 2010). We did not, however, observe a significant group improvement on any cognitive domain in our RCT (using the Repeatable Battery for Neuropsychological Status, RBANS) (Kulkarni et al., 2016). Similarly, a recent 16-week, double-blind randomised placebo-controlled trial in severely ill postmenopausal women with schizophrenia did not find significant cognitive improvements following 120 mg/day raloxifene on the Composite Brief Assessment of Cognition in Schizophrenia(Weiser et al., 2017). Cognitive enhancing effects of raloxifene in schizophrenia have, however, been demonstrated in several case studies (e.g. (Kulkarni et al., 2008; Usall et al., 2011) and other RCTs (e.g. Huerta-Ramos et al., 2014; Weickert et al., 2015).

Using a dose of 60 mg/day of adjunctive raloxifene for 12 weeks in postmenopausal women with schizophrenia (mean age of raloxifene group = 60.14), Huerta-Ramos et al (Huerta-Ramos et al., 2014) demonstrated a beneficial effect of raloxifene on verbal learning and phonemic fluency, with a small sample size of 26 (14 randomised to raloxifene). Another series of studies have looked at the effects of adjunctive raloxifene treatment (120 mg/day) in a younger mixed sample of males and females and adopted a 13-week randomised, double-blind, placebo controlled crossover design, with analyses conducted following the initial 6 week period (Weickert et al., 2015). This group first demonstrated significant improvements following 6 weeks of raloxifene (n = 40, mean age = 37.4) in immediate and delayed verbal learning (Wechsler Memory Scale Revised Logical Memory I and II) and attention/processing speed (Trail Making Test A) as compared to a placebo group (n = 39, mean age = 34.0). Supplementary analyses looking at the effects of sex reported that females, but not males showed an improvement in phonemic fluency (Controlled Oral Word Association Task), although the number of females and their age and menopause status was not reported.

Using the same 13-week cross-over design, this group has also demonstrated changes in neural activation during facial recognition and emotional inhibition tasks. Specifically raloxifene treatment (n = 10), relative to placebo (n = 10), was associated with significantly greater activation in the left inferior frontal gyrus and right hippocampus during angry face recognition, although there were no changes in performance accuracy (Ji et al., 2016). In a separate study, this group (Kindler et al., 2016) demonstrated that, relative to placebo, six weeks of adjunctive raloxifene treatment (120 mg/day) was associated with increased activity in the left PFC during inhibition of responses to negative words in a mixed male and female schizophrenia sample. They also reported a pharmacogenetics interaction, whereby performance accuracy and the fMRI BOLD in bilateral prefrontal cortex signal increased during inhibition of response to negative words specifically in the raloxifene group with the estrogen receptor-α gene ESR-1 rs9340799 A/A genotype. While this series of studies has demonstrated beneficial effects of 120 mg/day of raloxifene over a six week period on aspects of cognition, the results should be treated with caution given the mixed sex and mixed age sample.

The mixed findings of the effects of raloxifene on cognition in schizophrenia mirrors that found in the general population and is potentially a reflection of methodological differences and differences in participant characteristics. A recent systematic review and meta-analysis of the effects of raloxifene augmentation in schizophrenia failed to demonstrate a beneficial effect of raloxifene on cognition (de Boer et al., 2018); however, the authors concluded that raloxifene could potentially improve cognitive functioning in some cases and recommended further examination. Recent commentaries and reviews have suggested that the potential for hormone therapies, such as raloxifene, to improve cognition may be influenced by factors including treatment duration (de Boer et al., 2018), age and endogenous hormone levels (associated with changes in menopause status) (Bolton, 2016; de Boer et al., 2018).

The aim of the current study is to compare the efficacy of 120 mg/day adjunctive raloxifene for 12 weeks on cognitive performance in women with schizophrenia controlling for menopause status, stratifying women into pre-menopausal; peri-menopausal or post-menopausal status, in a pooled analysis using data from two clinical trials (where the primary aim of the clinical trials was to determine the effects of raloxifene on psychopathology).

2. Methods

2.1. Study design

The data for the present study represents pooled data from two clinical trials where the primary aim of both studies was to determine the effects of adjunctive raloxifene treatment on symptoms of psychosis on older women (Kulkarni et al., 2016) and younger, child bearing aged women with psychosis. In both clinical trials, women underwent assessment for eligibility in inpatient and outpatient settings in two treatment centres, Alfred Health and Barwon Health, in Melbourne, Australia, from January 1, 2006, to December 31, 2015. Both trials were conducted according to the CONSORT (Consolidated Standards of Reporting Trials) guidelines (Moher et al., 2010) and conducted as parallel-design, 12-week, double-blind RCT. Within each study, participants who passed screening were enrolled in the study, assigned by a computer-generated 1:1 block randomization schedule to receive 120 mg/d of raloxifene hydrochloride (administered as 1 capsule containing two 60-mg tablets [Evista; Eli Lilly]) or placebo (an identical capsule containing lactose [UNIVAR; Ajax Finechem]). Further details on study design can be found at Kulkarni et al (Kulkarni et al., 2016) (the psychopathology outcomes of the second clinical trial in pre-menopausal women are unpublished). The Alfred Human Research Ethics Committee approved both study protocols, and all participants gave written, informed consent before entering the study. Both studies are registered at ClinicalTrials.gov Identifier: NCT00361543 andNCT02354001.

2.2. Participants

For both clinical trials, women were eligible for the studies if they met DSM IV criteria for schizophrenia or schizoaffective disorder and were receiving a stable dose of antipsychotics for at least 4 weeks before enrolment. They were required to have normal findings on a mammogram within the last 12 months, and normal results of a Papanicolaou

Table 1a
Demographic and baseline clinical variables: Means (SD).

Variable	Raloxifene	Placebo	*P*
N	33	36	
Age (years)	46.09 (11.35)	47.17 (10.69)	0.69
Illness duration (years)	19.89 (10.43)	21.44 (10.30)	0.54
Risperidone –equivalent (mg)	7.56 (5.12)	9.18 (6.08)	0.26
Psychopathology	18.30 (4.87)	18.19 (3.65)	0.92
PANSS Positive	18.76 (4.21)	18.92 (4.99)	0.89
PANSS Negative			
Menopause status (*n*)	12	14	0.95
Premenopausal	7	7	
Perimenopausal	14	14	
Postmenopausal			
RBANS - baseline	23.09 (8.73)	22.31 (7.03)	0.68
List learning	11.48 (5.20)	12.14 (5.28)	0.61
Story memory	14.56 (4.44)	14.86 (3.50)	0.76
Figure copy	15.84 (3.58)	14.42 (4.61)	0.16
Line orientation	9.21 (0.99)	9.50 (0.74)	0.17
Picture naming	18.39 (5.71)	17.78 (5.31)	0.64
Semantic fluency	9.24 (2.00)	9.33 (2.58)	0.87
Digit span	38.73 (13.95)	33.36 (11.71)	0.09
Coding	4.18 (2.98)	4.61 (2.31)	0.50
List recall	18.48 (1.75)	18.92 (1.92)	0.33
List recognition	6.21 (3.27)	6.72 (3.27)	0.52
Story recall	8.88 (5.42)	8.58 (3.78)	0.79
Figure recall			
MADRS	16.42 (7.70)	20.86 (8.74)	0.03
Baseline	−5.20 (3.01)	−3.23 (11.03)	0.59
week 12#			

PANSS Positive and negative symptom scale; *Illness* duration schizophrenia syndrome duration; *pg/mL* pictograms per millilitre ng/ML nanograms per millilitre; MADRS Montgomery and Asberg Depression Rating Scale; #adjusted for baseline score (i.e. change from baseline to week 12).

test, breast and pelvic examination within the last 24 months. Females were not taking hormonal contraceptives or other forms of hormonal therapy and were able to commence the trial at any point in their menstrual cycle. For the purpose of the present study, 69 participants from the clinical trials who completed cognitive assessment at baseline and trial end (12 week) were included in the current analyses, randomized to raloxifene (36) or placebo (33). Demographic and clinical information for the participants included in the present study are displayed in Tables 1a and 1b.

2.3. Measures and procedure

Cognitive function was assessed at baseline and week 12 using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph et al., 1998). The RBANS is a brief, individually administered test designed to assess attention, language, visuospatial/constructional abilities, and immediate and delayed memory. It consists of 12 subtests that yield five index scores, with demonstrated reliability in schizophrenia (Wilk et al., 2002, 2004).

As part of the larger trials, participants' psychiatric and medical histories were recorded at screening, together with a physical examination and the Mini-International Neuropsychiatric Interview to confirm psychiatric diagnosis. Severity of depressive and psychotic illness was assessed at baseline and then fortnightly using the Montgomery and Asberg Depression Rating Scale(Montgomery and Asberg, 1979) and the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), respectively. Safety was assessed at baseline and each subsequent visit with the Adverse Symptoms Checklist of general and associated adverse effects. Levels of serum sex steroids and pituitary hormones (including estradiol, progesterone, luteinising hormone [LH] and follicle-stimulating hormone [FSH]) were assayed by the Alfred Pathology Service at baseline and weeks 4, 8, and 12using electrochemiluminescence method (ECLIA) or chemiluminescent microparticles immunoassay (CMIA). For the present study, sex steroid and pituitary hormone levels, in combination with clinical information, such as menstrual cycle regularity or final menstrual period, was used to classify women as premenopausal/reproductive; perimenopausal or postmenopausal according to the Stages of Reproductive Aging Workshop (STRAW) classification system (Harlow et al., 2012).

2.4. Analysis strategy

Baseline demographic variables and clinical characteristics were included for descriptive statistics of the sample: age (in years), illness duration (in years), antipsychotic medication use measured with Risperidone –equivalent doses (in mgs), MADRS and PANSS (positive, negative, general psychopathology) scores were measured as continuous variables. The menopause status strata variable was coded, 0 = premenopausal, 1 = perimenopausal, 2 = postmenopausal. The randomization ratio to each menopause stratum was 1:1. Measured levels of endogenous hormones (estrogen, progesterone, LH and FSH) were compared between treatments (raloxifene vs. placebo) by each menopause strata (treatment x menopause) at baseline and week 12 change (from baseline) values. In raloxifene effect estimation, we stratified for menopause status and adjusted for hormone levels (*a priori*) because we recently reported menopause status and hormone levels influence cognition in women with schizophrenia(Gurvich et al., 2018) and previous studies have suggested that age and endogenous hormones (which are both related to menopause status) may influence the potential for raloxifene to enhance cognition (Bolton, 2016; de Boer et al.,

Table 1b
Means (SE) for hormone variables stratified by menopause status, across treatment.

Variable	Pre-menopause			Peri-menopause			Post-menopause		
	Raloxifene	Placebo	P	Raloxifene	Placebo	P	Raloxifene	Placebo	P
Estrogen									
Baseline	224.3(38.0)	199.1(30.6)	0.61	228.9(68.0)	177.0(54.4)	0.55	86.3 (23.3)	76.4 (15.6)	0.70
Week 12#	**622.7(213.4)**	**16.4(45.5)**	**0.01**	326.8(189.3)	−68.2(53.3)	0.05	7.4(16.4)	62.2(60.0)	0.40
Progesterone									
Baseline	11.1(4.4)	5.1(1.6)	0.20	0.76 (0.2)	7.8(5.1)	0.17	1.1(0.1)	0.85(0.1)	0.20
Week 12#	−3.0(3.2)	0.28(1.6)	0.35	2.3 (2.1)	−6.6(5.1)	0.11	0.0(0.1)	0.2(0.1)	0.22
FSH									
Baseline	4.4(0.5)	6.0(1.1)	0.19	18.4 (5.6)	11.4(1.9)	0.23	67.7(7.9)	49.7(7.0)	0.09
Week 12#	2.7(1.0)	−0.33(1.1)	0.05	9.6(4.7)	13.8(3.5)	0.47	−8.0(6.6)	−4.1(2.5)	0.60
LH									
Baseline	3.6(0.5)	4.6(0.7)	0.36	10.2(3.1)	8.2(2.4)	0.60	19.1(2.7)	19.9(2.9)	0.83
Week 12#	**7.4(2.5)**	**1.4(1.1)**	**0.03**	7.5(3.9)	5.9(3.4)	0.75	2.2(2.5)	0.21(1.4)	0.48

Change from baseline hormone levels at week 12, *Bold* indicates statistical significance between treatments (raloxifene vs. placebo) at $p < 0.05$, SE standard error.

2018).

First, the null hypothesis test (NHT) of no raloxifene effect in each menopause category (i.e. pre-, peri-, and post-menopausal) was tested with a stratum-specific or stratified Wilcoxon rank-sum test, also known as van Elteren (Van Elteren, 1960). The van Elteren test is member of a family of Mantel-Haenszel mean score tests for change from baseline outcomes. We chose this test because its related asymptotic distribution is not influenced by the sample size of individual strata (Zhao, 2006). For the van Elteren test, the hypothesis was that raloxifene would result in better performance for cognition measures as compared to placebo (one-tailed p value). Second, we invoked the mean procedure in SAS to report the cognition outcome score differences (raloxifene vs. placebo) across each menopause stratum. Third, to covary for the effects of hormones on cognition outcomes, we modelled a nonparametric randomisation-based analysis of covariance (Koch et al., 1998). We compared the average treatment response on cognition outcomes by adjusting for hormone levels. The covariance analysis was stratified by menopause status variable (NParCov3 SAS/IML macro (Zink and Koch, 2012). All analyses were written with SAS/STAT/IML 9.4 (SAS institute, Cary NC).

3. Results

3.1. Demographic and baseline cognitive and clinical variables

Demographic and baseline cognitive and clinical variables for both groups; raloxifene and placebo are displayed in Table 1a. The age range of all participants (n = 69) was 21 to 77 years and the illness duration ranged from 2 to 44 years. As shown in Table 1a, the raloxifene and placebo groups did not significantly differ according to age, illness duration, antipsychotic medication, baseline PANSS scores or menopause status. The baseline MADRS scores were higher in the placebo group, but the MADRS change from baseline to week 12 did not differ between groups.As displayed in Table 1a, baseline performances did not differ significantly between the raloxifene and placebo groups on any of the RBANS subtest scores. RBANS index scores were not used in the analysis but were calculated to characterise the degree of cognitive impairment for this sample. The groups did not differ significantly on any of the RBANS index scores (all $ps > 0.05$). Mean index scores for the sample as a whole (n = 69) suggested Immediate Memory and Attention were classified in the “Borderline” range; Visuospatial/Construction and Delayed Memory were classified in the “Low Average” range and Language was placed at the lower end of the “Average” range. Collectively index scores demonstrated that the sample had cognitive impairment at baseline.

Table 1b presents baseline hormone levels and changes in hormone levels from baseline to week 12 for raloxifene and placebo groups by menopause strata. There were no significant differences in baseline hormone levels between raloxifene and placebo groups within each menopause strata. In relation to changes in hormone levels from baseline to week 12, the only changes that were observed were in the pre-menopausal stratum. The raloxifene group was associated with significantly greater increases in estrogen and LH levels from baseline to week 12, as compared to the placebo group.

The asymptotic p value (van Elteren test, Table 3) for each outcome indicates that after stratifying for menopause status, semantic fluency, picture naming and list recognition change from baseline scores for the raloxifene group differed significantly from placebo. Table 2 shows means (SD) of outcomes change scores for raloxifene vs. placebo, in each menopause stratum. For semantic fluency, mean change scores under raloxifene effect demonstrated an overall worsening of semantic fluency (although there was a benefit of raloxifene as compared to placebo within the perimenopause stratum). For picture naming, mean change scores under raloxifene effect was greater for all three strata. For list recognition, mean change scores were greater under raloxifene effect for the pre- and post- menopausal stratum. The remaining cognitive scores did not differ significantly between raloxifene and placebo.

Table 2
Summary statistics for cognitive change scores by menopause strata.

Outcome		Mean		SD	
	Strata	Raloxifene	Placebo	Raloxifene	Placebo
FC	Premenopausal	0.71	−1.33	2.7	2.5
	perimenopausal	−0.14	−0.57	3.4	2.5
	postmenopausal	0.09	0.07	4.4	3.6
SF	Premenopausal	−4.00	0.87	3.6	4.8
	Perimenopausal	−0.56	−2.57	2.6	4.0
	Postmenopausal	1.08	1.64	4.2	3.7
SM	Premenopausal	0.57	0.26	3.6	4.7
	Perimenopausal	5.28	1.42	4.8	6.6
	Postmenopausal	3.16	2.86	4.6	3.4
PN	Premenopausal	0.36	0.0	0.5	0.5
	Perimenopausal	0.29	0.0	0.7	0.6
	Postmenopausal	0.42	0.28	1.5	0.7
SR	Premenopausal	0.64	1.06	2.3	2.5
	Perimenopausal	3.14	1.00	3.4	3.3
	Postmenopausal	2.08	0.71	2.7	1.9
LR	Premenopausal	1.07	0.46	1.6	1.8
	Perimenopausal	0.0	0.29	2.3	3.1
	Postmenopausal	0.25	0.57	1.4	1.8
Lrec	Premenopausal	0.14	0.06	0.9	0.7
	Perimenopausal	0.29	0.28	1.6	0.9
	Postmenopausal	0.50	−0.64	1.8	1.2
DS	Premenopausal	0.64	0.60	2.1	3.5
	Perimenopausal	1.57	1.14	1.0	2.7
	Postmenopausal	−0.08	0.42	2.1	1.5
CD	Premenopausal	0.93	0.27	5.1	4.9
	Perimenopausal	−1.14	5.28	5.6	8.4
	Postmenopausal	1.25	3.07	6.4	5.3
LO	Premenopausal	0.0	−0.07	3.6	2.5
	Perimenopausal	0.14	−1.43	3.5	4.4
	Postmenopausal	−1.17	0.93	6.3	4.0
LL	Premenopausal	2.71	1.73	5.5	2.8
	Perimenopausal	−0.14	6.14	3.4	5.2
	Postmenopausal	2.41	3.71	5.4	6.2
FR	Premenopausal	1.36	1.07	2.8	3.3
	Perimenopausal	−0.43	−0.43	4.8	3.1
	Postmenopausal	1.75	3.21	3.4	3.3

SD *Standard Deviation. SF* semantic fluency; *LL* list learning; *SM* story memory; *FC* figure copy; *LO* line orientation; *PN* picture naming; *DS* digit span; *CD* coding; *LR* list recall; *Lrec* list recognition; *SR* story recall; *FR* figure recall.

Nonparametric randomisation-based analysis of covariance demonstrated a significant effect of raloxifene on semantic fluency performance as well as picture naming and list recognition. Specifically, raloxifene was associated with a worsening of semantic fluency (SF) performance ($\beta = -2.42$) and an improvement in list recognition (Lrec) and picture naming (PN) ($\beta = 0.62$ and $\beta = 0.39$, respectively).The reported standardised coefficients (Beta) for each outcome (Table 3: NonParCov test), allowed us to examine the relative magnitude of raloxifene effect size. For example, raloxifene dropped SF scores by almost two and a half standard deviations, while, increased Lrec and PN scores by three fifths and two fifths of a standard deviation, respectively. The criterion for covariate imbalance ($Q = 10.16$, $df = 4$) is borderline ($p = 0.05$), allowing us to state with caution that randomization provided a degree of equal distributions of covariates between raloxifene and placebo treatments.

4. Discussion

The primary conclusion that can be drawn from this study is that 12 weeks of adjunctive raloxifene, at a dose of 120 mg/day, altered

Table 3
Raloxifene effects on cognitive change scores (van Elteren and adjusted covariate analyses).

Outcome	van Elteren test		NonParCov test			
	CMH	P	Beta (β)	SE	95% CI	P
FC	2.03	0.07	1.05	0.75	−0.43 to 2.53	0.16
SF	2.70	**0.04**	−2.42	0.87	−4.15 to -0.71	**0.005**
SM	0.44	0.25	1.38	1.11	−0.80 to 3.56	0.21
PN	3.18	**0.03**	0.39	0.19	0.004 to 0.77	**0.04**
SR	1.30	0.12	0.63	0.65	−0.63 to 1.90	0.33
LR	0.11	0.35	−0.10	0.43	−0.95 to 0.74	0.81
Lrec	2.71	**0.04**	0.62	0.30	0.02 to 1.22	**0.04**
DS	0.001	0.50	−0.50	0.52	−1.52 to 0.54	0.35
CD	1.37	0.12	−1.65	1.43	−4.48 to 1.16	0.25
LO	0.75	0.21	0.15	0.88	−1.58 to 1.88	0.86
LL	0.92	0.16	−2.01	1.08	−4.13 to 0.11	0.06
FR	0.04	0.41	−0.57	0.84	−2.21 to 1.07	0.49

CMH Cochran –Mantel –Haenszel statistic, *SE* Standard Error, *SF* semantic fluency; *LL* list learning; *SM* story memory; *FC* figure copy; *LO* line orientation; *PN* picture naming; *DS* digit span; *CD* coding; *LR* list recall; *Lrec* list recognition; *SR* story recall; *FR* figure recall. *NonParCov* nonparametric analysis of covariance: adjusted for covariates (estrogen, progesterone, FSH and LH). Random covariates imbalance test Q = 10.16, DF = 4, $p = 0.05$. Both analyses stratified for menopause status.

performance on specific cognitive domains, dependent on menopause status. Raloxifene, as compared to placebo, was associated with a worsening of verbal fluency in pre- and post-menopausal women (although not in perimenopausal women). Verbal recognition memory benefits were associated with raloxifene in pre- and post- menopausal women and picture naming improved with raloxifene in all strata (pre, peri and post-menopause). We did not observe any other effects of raloxifene on cognitive performance.

Previous findings exploring the cognitive effects of raloxifene in both healthy and schizophrenia populations have been inconsistent. The findings from the current study suggest that menopause stage of the study population may partly explain some of these conflicting finding. The menopause transition is associated with fluctuating and eventually decreasing levels of ovarian estrogens (particularly estradiol) and progesterone and an increase in serum follicle stimulating hormone (FSH). While there is no consensus on the cognitive effects of the menopause transition, a meta-analysis (in non-clinical populations) concluded that the menopause transition is associated with decreases in verbal memory and verbal fluency (Weber et al., 2014) that potentially rebound to premenopausal levels in postmenopause (Greendale et al., 2011). The influence of menopause status on raloxifene's cognitive efficacy has not previously been investigated. Rodent models have suggested that raloxifene can act as a partial estrogen agonist and partial antagonist depending on the presence of estradiol (O'Neill et al., 2004) suggesting baseline hormone levels (particularly estradiol) are relevant in understanding the actions of raloxifene. Therefore, it is possible that baseline hormone levels and menopause stage could influence the efficacy of raloxifene on cognition.

An improvement in verbal learning and memory following raloxifene treatment is the most consistent finding in both healthy postmenopausal women (Jacobsen et al., 2010) and schizophrenia (Huerta-Ramos et al., 2014). In the current study, raloxifene, as compared to placebo, was associated with a significant improvement in verbal recognition memory (a component of verbal learning and memory). Mean change scores indicated this benefit was associated with women who were pre-menopausal and women who were post-menopausal. The two previous studies that have associated raloxifene with an improvement in verbal memory were in postmenopausal women. Huerta-Ramos et al (2014) using a lower dose of raloxifene (60 mg/day) for three months found a significantly greater improvement in verbal learning in the raloxifene group, as compared to a placebo group, in a post-menopausal group of women with schizophrenia. Jacobsen et al., (2010) also used a dose of 60 mg/day of raloxifene for 12 months in a group of non-clinical, late postmenopausal women, and found raloxifene treatment was associated with significant improvements in verbal memory (verbal recall), as compared to placebo. The current study found an improvement in verbal recognition memory, without any benefit in delayed verbal recall (or free recall). Different cognitive processes and neural pathways are associated with free recall as compared to verbal recognition performance (where cues or prompts are provided to aide retrieval) (e.g. (MacPherson et al., 2016). The lack of a broader improvement in verbal learning and memory (beyond recognition memory) may also be related to the dose used in the current study. Findings from previous studies suggest that a lower dose of raloxifene (60 mg) could be more beneficial for verbal memory, as compared to the 120 mg/day used in the current studies. In support of the dose dependency, rodent models provide preliminary evidence to suggest that the beneficial (estrogen agonistic) effects of raloxifene on hippocampal neurons, relevant to memory, may only be relevant within a narrow dose range (O'Neill et al., 2004).

The current results also demonstrated worsening of semantic fluency performance associated with raloxifene treatment, as compared to placebo, in pre- and post-menopausal women, with better performance for raloxifene as compared to placebo in peri-menopausal women. The one study (to our knowledge) that has reported an improvement in verbal fluency following raloxifene treatment in schizophrenia patients found an improvement in phonemic fluency, but not semantic fluency, in postmenopausal women (Huerta-Ramos et al., 2014). The current study only assessed semantic fluency (and not phonemic fluency) given that many studies have reported that individuals with schizophrenia have a specific impairment in semantic, but not phonemic fluency (Neill et al., 2014). However, results from the current study suggest raloxifene does not benefit semantic fluency and instead may actually impair performance in this domain for some groups of women.

Our third finding was that raloxifene was associated with an improvement in picture naming in all menopause stratum. This is the first study to report a beneficial effect of raloxifene treatment on a confrontation naming tasks, such as the RBANS picture naming, in women with schizophrenia. Improvements in similar naming tasks that have been associated with estrogen containing hormone therapy have been reported in healthy postmenopausal women (Lee et al., 2012) as well as in women with Alzheimer's disease (Henderson et al., 1996) (although a lack of association has also been reported (Pefanco et al., 2007)). The mechanisms by which raloxifene could improve confrontation naming are not clear, although could involve raloxifene effects on estrogen receptors or dopamine receptors in the CNS (Landry et al., 2002).

5. Limitations

There are several limitations of this study that must be acknowledged. As this study was essentially post-hoc analyses based on pooled data (where cognition was not the primary outcome measure of the initial studies), the RBANS was the only measure of cognition. Although the RBANS provides a good measure of general cognition, the findings from this study do not allow for comparisons with other studies that have found raloxifene related improvements in specific areas of cognition related to emotion face recognition (Ji et al., 2016) and visual processing/visual attention (Weickert et al., 2015). We also had a smaller sample size of women within the perimenopausal stratum. However, the related asymptotic distributions of van Elteren test are not influenced by the sample size of individual strata (the test asymptotic p values are reliable). The analysis of covariance was nonparametric, which means no estimates for covariates or strata were available. However, primarily, our main focus aimed at reporting covariate-adjusted treatment effects. Further, adjusted effects are associated with greater power and efficiency relative to unadjusted. Also, the nonparametric analysis was relatively assumptions-free. Finally, illness duration and age are closely related to menopause status and the

individual contributions of age and illness duration to the cognitive effects of raloxifene could potentially be explored in future research with larger sample sizes using parametric methods.

6. Conclusions

In conclusion, our key finding, in a sample of 69 women with schizophrenia was that raloxifene treatment at a dose of 120 mg/day for 12 weeks alters specific cognitive domains after stratifying for menopause status and adjusting for endogenous hormones levels. Raloxifene related benefits were observed for verbal recognition memory in pre- and post-menopausal women. Semantic fluency worsened following raloxifene treatment in pre- and postmenopausal women. Picture naming benefits associated with raloxifene treatment were observed across all menopause stratum. We did not observe any effects of raloxifene on the other RBANS tasks. Additional benefits of raloxifene treatment for cognition in schizophrenia have been reported at 60 mg/day (Huerta-Ramos et al., 2014). In contrast, treatment for psychopathology symptoms of schizophrenia in women, appears to be more beneficial with 120 mg/day adjunctive raloxifene (Kulkarni et al., 2016, 2010). Hence, future research should compare raloxifene dosing and the effects on cognition and psychopathology within each menopause stage. The findings from the current study highlight the importance of considering menopause status when interpreting the effects of hormonal treatments.

Conflict of interest

The authors declare that they have no conflict of interest

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