Psychiatry Research 215 (2014) 540–546

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Minocycline add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: Randomized double-blind placebo-controlled study

Mohammad-Reza Khodaie-Ardakani [a], Omid Mirshafiee [b], Mehdi Farokhnia [b], Masih Tajdini [b], Seyed-Mohammad-Reza Hosseini [b], Amirhossein Modabbernia [b], Farzin Rezaei [c], Bahman Salehi [d], Habibeh Yekehtaz [b], Mandana Ashrafi [b], Mina Tabrizi [e], Shahin Akhondzadeh [b,*]

[a] Razi Psychiatric Hospital, Welfare Sciences University, Tehran, Iran
[b] Psychiatric Research Centre, Roozbeh Psychiatric Hospital, Tehran University of Medical Sciences, South Kargar Street, Tehran 13337, Iran
[c] Department of Psychiatry, Kurdistan University of Medical Sciences, Sanandaj, Iran
[d] Department of Psychiatry, Arak University of Medical Sciences, Arak, Iran
[e] Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 18 November 2012
Received in revised form
23 August 2013
Accepted 28 December 2013
Available online 9 January 2014

Keywords:
Glutamate
Minocycline
Negative symptoms
Schizophrenia

ABSTRACT

The objective of this study was to assess the efficacy and tolerability of minocycline add-on to risperidone in treatment of negative symptoms of patients with chronic schizophrenia. In a randomized double-blind placebo-controlled study, 40 patients with chronic schizophrenia who were stabilized on risperidone for a minimum duration of eight weeks were recruited. The patients were randomly assigned to minocycline (titrated up to 200 mg/day) or placebo in addition to risperidone (maximum dose of 6 mg/day) for eight weeks. Positive and Negative Syndrome Scale (PANSS), Hamilton Depression Rating Scale, and Extrapyramidal Syndrome Rating Scale were used. Thirty-eight patients completed the study. Significant time × treatment interaction for negative [$F(2.254,85.638)=59.046$, $P<0.001$] general psychopathology [$F(1.703,64.700)=6.819$, $P=0.001$], and positive subscales [$F(1.655,62.878)=5.193$, $P=0.012$] as well as total PANSS scores [$F(1.677,63.720)=28.420$, $P<0.001$] were observed. The strongest predictors for change in negative symptoms were the treatment group ($\beta=-0.94$, $t=-10.59$, $P<0.001$) followed by the change in PANSS positive subscale ($\beta=-0.185$, $t=-2.075$, $P=0.045$). Side effect profiles of the two treatment regimens were not significantly different. Minocycline seems to be an efficacious and tolerable short-term add-on to risperidone for treatment of negative and general psychopathology symptoms of schizophrenia.

1. Introduction

Dopaminergic system imbalance is the most widely studied mechanism in pathophysiology of schizophrenia. Blocking the dopamine receptors by means of current antipsychotics mostly relieves positive symptoms with limited effects on negative or cognitive symptoms (Murphy et al., 2006). Atypical antipsychotics have been reported to be effective in treating negative symptoms although their effect is suboptimal (Murphy et al., 2006). Nevertheless, recent studies do not support the original claims of “atypical antipsychotics having beneficial effects on negative symptoms in schizophrenia” (National Institute for Clinical Excellence, 2009; Leucht et al., 2009). Evidence suggests that in addition to dopaminergic pathways, other neurotransmitter mechanisms including serotoninergic and glutamatergic as well as inflammatory and oxidative pathways might be implicated in the pathophysiology of negative and cognitive symptoms of schizophrenia (Tuominen et al., 2005; Murphy et al., 2006).

Minocycline is a second-generation brain-penetrable tetracycline with antimicrobial and anti-inflammatory effects as well as N-Methyl-D-Aspartate (NMDA) receptor modulating properties (Macdonald et al., 1973; Chaves et al., 2009). Neuroprotective effects of minocycline were first seen in studies on mouse model of Huntington′s disease (Berger, 2000) and amyotrophic lateral sclerosis (Zhang et al., 2003) in which minocycline delayed mortality and prevented disease progression. Subsequently, minocycline was shown to be effective in neurological diseases such as Parkinson′s disease (Du et al., 2001) and ischemia (Yrjanheikki et al., 1999) in humans. In one case report, several psychiatric

* Corresponding author. Tel.: +98 21 88281866; fax: +98 21 55419113.
E-mail address: s.akhond@neda.net (S. Akhondzadeh).

0165-1781/$ - see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psychres.2013.12.051

symptoms of a patient with Huntington's disease were improved by minocycline administration (Denovan-Wright et al., 2002). A study has shown that microglial activation and proliferation and nitric oxide synthesis is inhibited by minocycline (Miyaoka et al., 2008).

Treatment of schizophrenia still remains a challenge. Anti-inflammatory drugs such as celecoxib have been useful in treating symptoms of schizophrenia (Akhondzadeh et al., 2007). NMDA receptor of glutamate has been of increasing interest as a target for treatment of negative and cognitive symptoms of schizophrenia (Rezaei et al., 2013). Several studies have suggested that drugs with action on NMDA receptors might be effective in the treatment of schizophrenia (Rezaei et al., 2013; Farokhnia et al., 2013). A number of studies suggest that minocycline prevents the neurotoxic effects of NMDA antagonists and may exert a differential effect on NMDA receptor signaling pathways (Zhang et al., 2007; Fujita et al., 2008). In one study, administration of minocycline for patients with catatonic schizophrenia showed encouraging results (Ahuja and Carroll, 2007). In an open label study, efficacy of minocycline in schizophrenia was assessed. This study showed that minocycline can be an effective agent for treating schizophrenia (Miyaoka et al., 2008). Two randomized double-blind placebo-controlled clinical trials (RCTs) investigated the effect of minocycline on negative symptoms of early-stage schizophrenia. The results showed that combination of minocycline with a standard treatment of schizophrenia significantly improves the negative symptoms (Levkovitz et al., 2010; Chaudhry et al., 2012). The efficacy of minocycline treatment in patients with chronic stable schizophrenia has not been investigated yet.

We hypothesized that minocycline add-on to risperidone can play a beneficial role in the reduction of primary negative symptoms in patients with chronic schizophrenia. This study assessed the adjunctive effect of minocycline to risperidone as a popular atypical antipsychotic on negative symptoms of patients with stable chronic schizophrenia.

2. Methods

2.1. Trial design and setting

This was an eight-week, double-center, randomized, double-blind, placebo-controlled, parallel-group trial. Each patient was evaluated at baseline visit and at weeks 2, 4, 6, and 8. The study was authorized by the institutional review board of Tehran University of Medical Sciences (TUMS) (Grant no.: 11921), performed in accordance with the Declaration of Helsinki, and approved by the ethics committee at TUMS. Written informed consent was obtained from the eligible participants and their legal representative before entering the study and the patients were informed about their right to withdraw from the study anytime they wish. This trial was registered in the Iranian Clinical Trials Registry (IRCT201202241556N34; www.irct.ir)

2.2. Participants

Male and female outpatients aged 18–50 years were eligible to participate in this study if they had a diagnosis of schizophrenia based on the DSM IV-TR criteria (American Psychiatric Association., 2000) and a minimum disease duration of two years. Diagnosis was based on Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID) and was confirmed with chart review and senior physician interview. Moreover, the eligible patients were required to be treated with a stable dose of risperidone for a minimum of eight weeks and had to be clinically stable for at least four weeks before the study. Clinical stability was defined as $\leq 20\%$ total score change on two consecutive ratings on the positive and negative syndrome scale (PANSS) (Kay et al., 1987). Patients with significant depression, defined as a score ≥ 14 on the 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) or a score of ≥ 4 on depression item of PANSS, were excluded from the study. Other exclusion criteria were serious medical or neurological disorders, any other psychiatric disorder on axis I, alcohol or substance (other than nicotine) dependence, mental retardation (based on clinical judgment), history of hypersensitivity to minocycline, pregnancy, lactation, and hepatic or kidney disease. Women in reproductive age were included only if they were using a reliable contraception method. Patients were also excluded if they had received electroconvulsive therapy (ECT) during the last two weeks. Patients were not allowed to use antidepressants, mood stabilizers, or a second antipsychotic (as an augmentative strategy) during the course of the trial.

2.3. Study settings

The study was conducted in outpatient general psychiatry clinics of Roozbeh psychiatric hospital (Tehran University of Medical Sciences, Tehran, Iran) and Razi Hospital (Welfare Sciences University, Tehran, Iran) from March to October 2012. There were no ethnical or regional restrictions for participants as they were referred from different parts of Tehran and different regions of Iran as long as the patients and their families could adhere to the trial plan.

2.4. Interventions

Eligible patients were randomized into two groups to receive risperidone (Risperdal, Janssen Pharmaceuticals) plus either minocycline or placebo for eight weeks. The dose of risperidone was 4–6 mg/day during the course of the trial. Minocycline initial dosage was 100 mg/day for the first week followed by 200 mg/day for the subsequent seven weeks. Patients did not receive any behavior intervention therapy during the course of the trial.

2.5. Outcomes

The efficacy assessment measure in this study was the PANSS. This is a 30-item rating scale which has been widely used for measuring the severity of symptoms in patients with schizophrenia and has been applied in several studies in Iran (Ghaleiha et al., 2010; Akhondzadeh et al., 2011; Arbabi et al., 2012). It consists of validated subscales to examine positive (7 items), negative (7 items) and general psychopathological (16 items) symptoms of schizophrenia. These three subscales are summed up in the PANSS total score (Kay et al., 1987). Patients were rated by PANSS based on a structured clinical interview at weeks 0, 2, 4, 6, and 8 following the baseline/screening session. In addition, HDRS was administered at baseline and week 8 in order to assess changes in depressive symptoms. This clinician-rated scale contains 17 questions (measured either on 5-point or 3-point scales) which assess the severity of depression-related symptoms (Hamilton, 1960). The difference in the PANSS negative subscale score decrease from baseline to week 8 between the two groups was the primary outcome measure in this study. The difference between the two study groups on the basis of changes in other PANSS subscales and the PANSS total score was considered as secondary outcome measures. Four trained raters were responsible for rating the patients with an inter-reliablity of $>90\%$ on PANSS total symptoms.

2.6. Side effects

Patients were encouraged to inform the research team about any unexpected symptom after entering the study. Side effects were recorded at each visit using a subjective 25-item checklist covering a broad range of complaints. Extrapyramidal Symptoms Rating Scale (ESRS) (part one: parkinsonism, dystonia, dyskinesia; sum of 11 items) (Chouinard and Margolese, 2005) was administered at baseline and week 8 in order to assess the extrapyramidal symptoms. The behavioral and side effects appraisals were completed by independent raters. A thorough physical examination was performed and vital signs were recorded at the screening session and each post-baseline visit.

2.7. Sample size

Assuming a difference of 3 between the two groups of the trial on the PANSS negative subscale, a standard deviation (S.D.) of 3, a two-tailed significance of 0.05, and a power of 80%, a sample size of 32 were calculated (based on our pilot study). Forty patients were planned for recruitment with a 20% drop-out rate assumption.

2.8. Randomization, allocation concealment and blinding

A computer-generated code was used in order to randomly assign the patients to minocycline or placebo group in a 1:1 ratio. The assignments were kept in sequentially numbered sealed, opaque envelopes until the end of the study. The patients and the psychiatrists who referred them were blind to assignments as well as the rater and the person who administered the medications. Different persons were responsible for random allocation and rating of the patients. Placebo was identical in appearance (shape, size, color, and taste) to minocycline and was dispensed by the investigational drug pharmacist.

2.9. Statistical methods

Data was analyzed by IBM SPSS Statistic 20 (IBM Corporation). Fisher's exact test was used to compare the baseline characteristics as well as the frequency of side effects between the two study groups. Independent sample *t*-test and Cohen's d effect size was used for analysis of the difference between two groups in the baseline scores and the change in each measurement (PANSS, HDRS, and ESRS) score from baseline to the study endpoint. The effect of time and time × treatment interaction was assessed by two-factor repeated measure Analysis of Variance (ANOVA). If Mauchly's test of sphericity was significant, Greenhouse–Geisser correction for degrees of freedom was used. Multiple linear regression analysis was used to predict the change in PANSS negative subscale by assigning the change in positive subscale, HDRS, and ESRS scores as well as the treatment group. Categorical variables were described in number (%) and continuous variables as mean ± S.D. Mean differences between the two groups were reported as MD (95% confidence intervals, 95%CI). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Participants

Sixty-four patients were screened for eligibility; 40 patients were randomly assigned to two groups; and 38 patients (minocycline $n=20$, placebo $n=18$) completed the trial (Fig. 1). Patients with a baseline and at least one post-baseline assessment were included in the analysis based on a Last Observation Carried Forward (LOCF) algorithm (total $n=40$). There was no significant difference between baseline characteristics of patients in the two groups (Table 1).

3.2. PANSS negative subscale

There was no significant difference between the two groups in the PANSS negative subscale scores at baseline [MD(95%CI)=0.10 (−1.61 to 1.81), $t(38)=0.118$, $P=0.907$]. Two-factor repeated measure ANOVA showed significant effect for time [Greenhouse–Geisser corrected: $F(2.254,85.638)=65.246$, $P<0.001$] as well as time × treatment interaction [Greenhouse–Geisser corrected: $F(2.254,85.638)=59.046$, $P<0.001$] (Fig. 2). The minocycline group demonstrated significantly greater improvement in the negative symptoms than the placebo group by the end of the trial [MD(95%CI)=4.650(3.74–5.55), $t(38)=10.429$, $P<0.001$]. When

Table 1
Baseline characteristics of the patients.

Variable	Minocycline + risperidone (*n*=20)	Placebo + risperidone (*n*=20)
Gender (%)		
Male	14(70%)	15(75%)
Female	6(30%)	5(25%)
Age (years), mean ± S.D.	41.05 ± 7.47	38.95 ± 7.78
Marital status (%)		
Single	15(75%)	13(65%)
Married	5(25%)	6(30%)
Divorced	–	1(5%)
Level of education (%)		
Illiterate	2(10%)	–
Primary school	12(60%)	16(80%)
High school diploma	5(25%)	4(20%)
University degree	1(5%)	–
Smoking (%)	16(80%)	18(90%)
Risperidone dose (mg), mean ± S.D.	4.40 ± 0.52	4.30 ± 0.62
Weight (Kg), mean ± S.D.	78.45 ± 10.45	79.92 ± 11.15
Duration of illness (years), mean ± S.D.	20.90 ± 8.02	18.75 ± 7.55
Types of schizophrenia (%)		
Paranoid	8(40%)	9(45%)
Residual	8(40%)	7(35%)
Disorganized	3(15%)	4(20%)
Undifferentiated	1(5%)	–

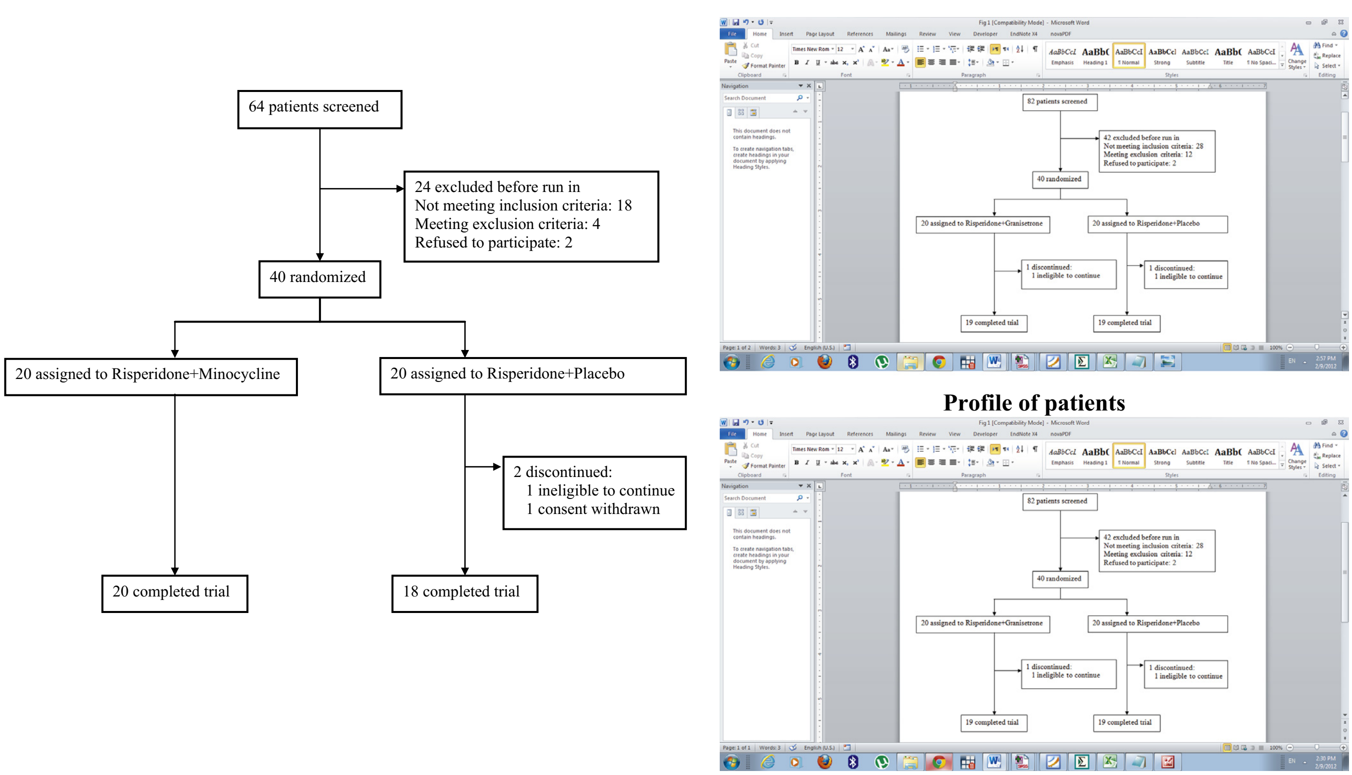

Fig. 1. Flow diagram of the trial.

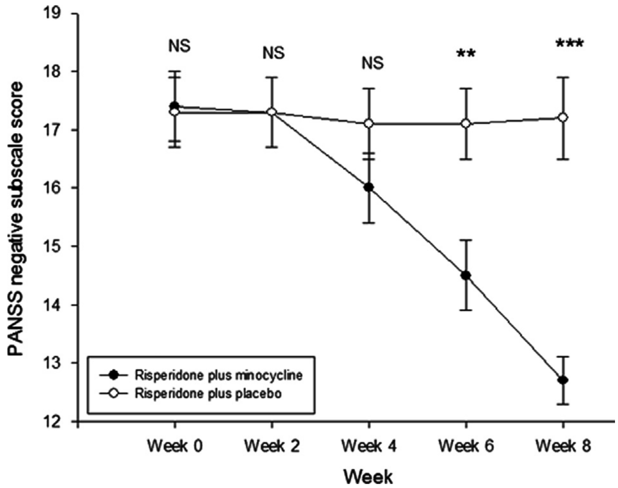

Fig. 2. Results of repeated measure analysis of variance for comparison of the effects of two treatments on negative subscale score of PANSS between the two study groups. Values represent mean ± SEM. *P* values show the result of independent sample *t*-test for comparison of the score change from the baseline between the two groups at each time point. $^{**}P<0.01$, $^{***}P<0.001$, and ns: non-significant.

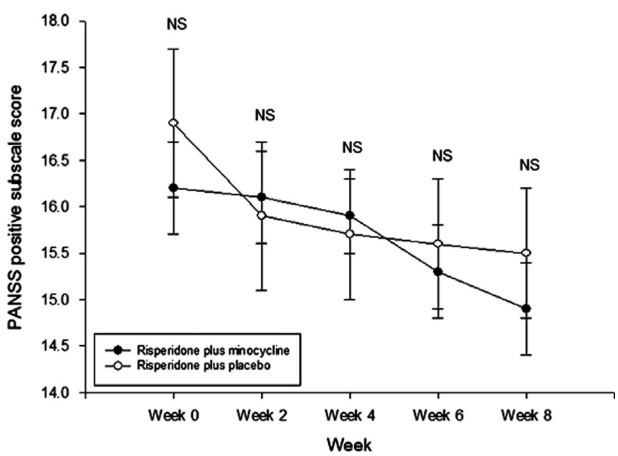

Fig. 3. Results of repeated measure analysis of variance for comparison of the effects of two treatments on positive subscale score of PANSS between the two study groups. Values represent mean ± SEM. *P* values show the result of independent sample *t*-test for comparison of the score change from the baseline between the two groups at each time point. ns: Non-significant.

change in PANSS negative subscale was predicted by multiple linear regression analysis, it was found that the treatment group ($\beta=-0.94$, $t=-10.59$, $P<0.001$) and the change in PANSS positive subscale ($\beta=-0.185$, $t=-2.075$, $P=0.045$) were independent significant predictors. Change in the HDRS ($\beta=0.036$, $t=0.438$, $P=0.664$) and ESRS ($\beta=0.028$, $t=0.346$, $P=0.731$) scores could not significantly predict the change in PANSS negative subscale scores. The strongest predictor of change in the negative syndrome was the treatment group as indicated by very large standardized beta coefficient.

3.3. PANSS positive subscale

PANSS positive subscale scores were not significantly different between the two groups at baseline [MD(95%CI)=0.30 (−1.58 to 2.18), $t(38)=0.322$, $P=0.750$]. Two-factor repeated measure ANOVA demonstrated significant effect for time [Greenhouse–Geisser corrected: $F(1.655,62.878)=18.638$, $P<0.001$] and also for time × treatment interaction [Greenhouse–Geisser corrected: $F(1.655,62.878)=5.193$, $P=0.012$] (Fig. 3). Improvement in positive subscale scores by week 8 was significantly different between the two groups [MD(95%CI)=0.90 (0.24–1.55), $t(38)=2.791$, $P=0.008$].

3.4. PANSS general psychopathology subscale

Similar baseline PANSS general psychopathology subscale scores were noted between two groups [MD(95%CI)=−1.10 (−4.77 to 2.57), $t(38)=-0.606$, $P=0.548$]. Two-factor repeated measure ANOVA determined significant effect for time [Greenhouse–Geisser corrected: $F(1.703, 64.700)=6.819$, $P=0.003$] and time × treatment interaction [Greenhouse–Geisser: $F(1.703, 64.700)=6.819$, $P=0.001$] (Fig. 4). Significantly more improvement in the general psychopathology symptoms was observed in the minocycline group than the placebo group by week eight [MD(95%CI)=2.70 (0.83–4.56), $t(38)=2.930$, $P=0.006$].

3.5. PANSS total score

Baseline PANSS total scores were not significantly different between two groups [MD(95%CI)=−0.55(−4.38 to 3.28), $t(38)=-0.290$, $P=0.773$]. In two-factor repeated measure ANOVA, the effect of time was significant [Greenhouse–Geisser corrected: $F(1.677,63.720)=45.660$, $P<0.001$]. Behavior of the two groups was significantly different across time as indicated by the effect of time × treatment interaction [Greenhouse–Geisser corrected: $F(1.677,63.720)=28.420$, $P<0.001$] (Fig. 5). By week eight, significantly higher reductions in PANSS total scores were observed in the minocycline group compared with the placebo group [MD(95%CI)=8.95(6.05–11.84), $t(38)=6.263$, $P<0.001$].

3.6. Hamilton Depression Rating Scale

At baseline, HDRS scores did not significantly differ between the two groups [MD(95%CI)=−0.10 (−0,78 to 0.58), $t(38)=-0.297$, $P=0.768$]. No significant difference was noticed in reduction of HDRS scores between the two groups by the study endpoint [MD(95%CI)=−0.05(−0.51 to 0.41), $t(38)=-0.216$, $P=0.830$].

3.7. Extrapyramidal Symptoms Rating Scale

Baseline ESRS scores were similar between the two groups [MD(95%CI)=−0.15(−0.78 to 0.48), $t(38)=0.474$, $P=0.637$]. There was

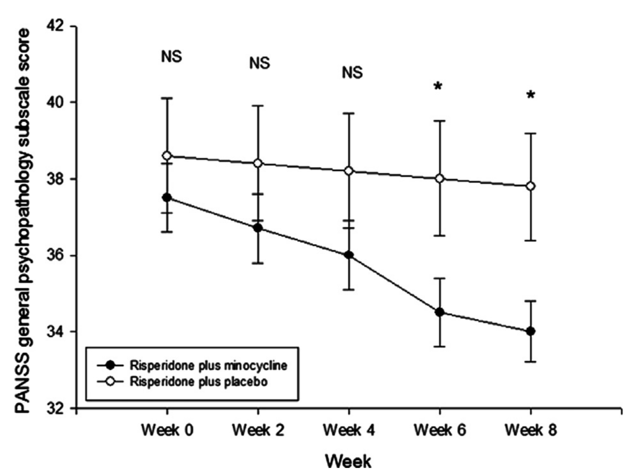

Fig. 4. Results of repeated measure analysis of variance for comparison of the effects of two treatments on general psychopathology subscale score of PANSS between the two study groups. Values represent mean ± SEM. *P* values show the result of independent sample *t*-test for comparison of the score change from the baseline between the two groups at each time point. $^{*}P<0.05$ and ns: non-significant.

no significant difference in the change of ESRS scores from baseline to week eight between the two study groups [MD(95%CI)= −0.10(−0.77 to 0.57), $t(38)=-0.300$, $P=0.766$] (Table 2).

3.8. Clinical complications

Other than extrapyramidal symptoms assessed by ESRS, 19 side effects were observed over the course of the trial based on the side effects checklist. The most frequent complaint of the patients was daytime drowsiness, although no significant difference was detected between the two groups in the frequency of side effects (Table 3).

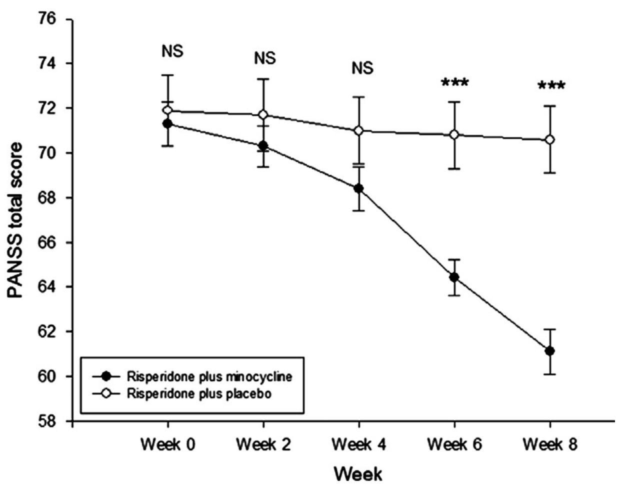

Fig. 5. Results of repeated measure analysis of variance for comparison of the effects of two treatments on total score subscale score of PANSS between the two study groups. Values represent mean ± SEM. *P* values show the result of independent sample *t*-test for comparison of the score change from the baseline between the two groups at each time point. $^{***}P<0.001$ and ns: non-significant.

4. Discussion

According to our study, minocycline is beneficial for treating primary negative and general psychopathology of schizophrenia when it is used as an adjunct to risperidone. When primary negative symptoms are assessed in clinical trials, effects of changes in other symptoms on negative symptoms should be considered (Kirkpatrick et al., 2006; Murphy et al., 2006; Buchanan, 2007). These factors include changes in positive, depressive and extrapyramidal symptoms throughout the course of the study. The only way to solve this problem is stabilizing the patients sufficiently prior to the trial to minimize the confounding effect of positive symptoms. Also, the efficacy of an antipsychotic drug on negative symptoms is not interpretable unless changes in depressive and extrapyramidal symptoms during the course of the trial are minimized or at least controlled.

In the present study, the patients were adequately stabilized and changes in the positive, depressive, and extrapyramidal symptoms were controlled while analyzing the effect of treatment on negative symptoms. Thus, we can attribute the improvement of negative symptoms in our patients to reduction of their primary negative symptoms. Two RCTs have previously shown beneficial effects of minocycline on negative symptoms of the patients with early-stage schizophrenia (Levkovitz et al., 2010; Chaudhry et al., 2012). In the mentioned RCTs, the patients had higher PANSS scores and thus were less stable, had shorter duration of illness (<5 year), and longer duration of add-on therapy (>5 months). There was a three point reduction in negative symptoms in our study comparable to four to five point reduction in a longer time of the two other RCTs. Importantly, our patients had an average disease duration of 20 years which potentially decreases the possibility of treatment response.

Mechanisms of antipsychotic action of minocycline have not been clearly defined yet. However, evidence suggests that it prevents the neurotoxic effects of NMDA antagonists and may exert a differential effect on NMDA receptor signaling pathways (Zhang et al., 2007; Fujita et al., 2008). Hence, it has been postulated that

Table 2
Mean ± S.D. of the two treatment arms on different measures of the study.

Measure	Week	Minocycline+risperidone ($n=20$)	Placebo+risperidone ($n=20$)	$t(38)$	*P* value
PANSS negative subscale (mean ± S.D.)	0	17.45 ± 2.79	17.35 ± 2.56	0.118	0.907
	2	17.30 ± 2.67	17.35 ± 2.56	−0.060	0.952
	4	16.00 ± 2.67	17.10 ± 2.67	−1.301	0.201
	6	14.55 ± 2.54	17.15 ± 2.79	−3.076	0.004
	8	12.70 ± 2.02	17.25 ± 3.00	−5.610	<0.001
PANSS positive subscale (mean ± S.D.)	0	16.25 ± 2.19	15.95 ± 3.54	0.322	0.750
	2	16.15 ± 2.13	15.90 ± 3.49	0.273	0.786
	4	15.90 ± 1.94	15.75 ± 3.32	0.174	0.863
	6	15.30 ± 2.47	15.65 ± 3.31	−0.379	0.707
	8	14.80 ± 2.26	15.50 ± 3.17	−0.688	0.495
PANSS general psychopathology subscale (mean ± S.D.)	0	37.50 ± 4.17	38.60 ± 6.96	−0.606	0.548
	2	36.75 ± 4.11	38.45 ± 6.90	−0.946	0.350
	4	36.05 ± 3.91	38.20 ± 6.59	−1.254	0.217
	6	34.55 ± 3.83	38.00 ± 6.52	−2.038	0.049
	8	34.05 ± 3.53	37.85 ± 6.38	−2.329	0.025
PANSS total score (mean ± S.D.)	0	71.35 ± 4.54	71.90 ± 7.14	−0.290	0.773
	2	70.35 ± 4.02	71.70 ± 7.09	−0.740	0.464
	4	68.40 ± 4.48	71.05 ± 6.87	−1.444	0.157
	6	64.45 ± 3.77	70.80 ± 6.88	−3.616	0.001
	8	61.10 ± 4.68	70.60 ± 6.90	0.039	<0.001
HDRS (mean ± S.D.)	0	8.10 ± 1.02	8.20 ± 1.10	−0.297	0.768
	8	8.00 ± 0.91	8.05 ± 1.05	−0.160	0.873
ESRS (mean ± S.D.)	0	1.65 ± 1.08	1.80 ± 0.89	−0.476	0.637
	8	1.40 ± 0.68	1.45 ± 0.60	−0.246	0.807

ESRS: Extrapyramidal Symptoms Rating Scale; HDRS:Hamilton Depression Rating Scale; and PANSS: Positive and Negative Syndrome Scale.

Table 3
Frequency of side effects in the two study groups.

Side effect	Minocycline+risperidone (n=20)	Placebo+risperidone (n=20)	P value
Daytime drowsiness (%)	3 (15%)	6 (30%)	0.50
Morning drowsiness (%)	9 (45%)	8 (40%)	1.00
Constipation (%)	4 (20%)	5 (25%)	1.00
Dizziness (%)	4 (20%)	2 (10%)	0.66
Stiffness (%)	–	3 (15%)	0.20
Slowed movement (%)	6 (30%)	6 (30%)	1.00
Tremor (%)	3 (15%)	4 (20%)	1.00
Increased appetite (%)	3 (15%)	4 (20%)	1.00
Nervousness (%)	–	1 (5%)	1.00
Restlessness (%)	3 (15%)	4 (20%)	1.00
Urinary retention (%)	1 (5%)	–	1.00
Loss of appetite (%)	3 (15%)	–	0.20
Fatigue (%)	4 (20%)	6 (30%)	0.71
Diarrhea (%)	4 (20%)	2 (10%)	0.66
Dry mouth (%)	6 (30%)	3 (15%)	0.20
Trouble swallowing (%)	1 (5%)	–	1.00
Sore throat/tongue (%)	3 (15%)	–	1.00
Abdominal pain (%)	1 (5%)	1 (5%)	1.00
Tachycardia (%)	–	1 (5%)	1.00

the effects of minocycline on the brain may be partially exerted through NMDA receptor modulation. The differential (synaptic versus extra synaptic) modulation of NMDA receptor signaling may be the mechanism by which minocycline mediates its neuroprotective effect (Chaves et al., 2009). Recently, minocycline has been considered as a positive modulator of the GluR1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. An AMPA antagonist can be a neuroprotective agent by increasing the level of brain derived neurotrophic factor (Kantrowitz and Javitt, 2010). Furthermore, minocycline controls the NMDA receptor signaling by inhibiting p38 MAPK and activating phosphoinositide-3-kinase/Akt. Glutamate facilitates the cortico-cortical and total brain neurons transmissions (Pi et al., 2004). Moreover, reducing microglial activation by direct or indirect effect on cell division cycle is another important mechanism of action of minocycline (Tsai et al., 2004). In vitro experiments demonstrated that activation and proliferation of cultured microglia is influenced by minocycline (Tuominen et al., 2005), an effect that has been linked to glutamate excitotoxicity (Tsai et al., 2004). Apoptosis is induced by caspase 9 and 3 which are activated by transition of cytochrome c into the cytosol (Zhang et al., 2007). Minocycline inhibits this transition by maintaining mitochondrial membrane. Other in vitro evidence has shown the role of minocycline in increasing antiapoptotic factor Bcl-2. Therefore, minocycline can also be considered as an anti-apoptotic agent (Wang et al., 2003, 2004). Destruction of lipids and proteins in the cell membrane is caused by free radicals. Minocycline suppressed generation of free radicals produced by leukocytes and inhibited T-cell proliferation cultured from human fetal calf serum. Hence, minocycline can also protect cell membrane integrity against free radicals (Amin et al., 1996). We emphasize however, that with respect to the short duration of treatment and long duration of illness in our patients it is very unlikely that minocycline has exerted its therapeutic effect through changes in apoptotic or radical scavenger pathways.

Small sample size and short duration of therapy were two limitations of our study. Moreover, lack of specialized cognitive measures and probably functional neuroimaging studies warrants further effort for a more in-depth investigation of minocycline therapeutic effect on symptoms of chronic schizophrenia.

4.1. Conclusion

The present study showed that minocycline seems to be an efficacious and tolerable add-on to risperidone in treating negative and general psychopathological symptoms of patients with chronic schizophrenia.

Acknowledgment

This study was Dr. Omid Mirshafiee's postgraduate thesis toward the Iranian Board of Psychiatry. This study was supported by a Grant from Tehran University of Medical Sciences to Prof. Shahin Akhondzadeh (Grant no.: 11921). The trial was registered in the Iranian registry of clinical trials (IRCT201202241556N34; www.irct.ir).

References

Ahuja, N., Carroll, B.T., 2007. Possible anti-catatonic effects of minocycline in patients with schizophrenia. Progress in Neuropsychopharmacology and Biological Psychiatry 31, 968–969.
Akhondzadeh, S., Ghayyoumi, R., Rezaei, F., Salehi, B., Modabbernia, A.H., Maroufi, A., Esfandiari, G.R., Naderi, M., Ghebleh, F., Tabrizi, M., Rezazadeh, S.A., 2011. Sildenafil adjunctive therapy to risperidone in the treatment of the negative symptoms of schizophrenia: a double-blind randomized placebo-controlled trial. Psychopharmacology (Berl) 213, 809–815.
Akhondzadeh, S., Tabatabaee, M., Amini, H., Ahmadi Abhari, S.A., Abbasi, S.H., Behnam, B., 2007. Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. Schizophrenia Research 90, 179–185.
American Psychiatric Association, 2000. Diagnostic Criteria from DSM-IV-TR. American Psychiatric Association, Washington, D.C.
Amin, A.R., Attur, M.G., Thakker, G.D., Patel, P.D., Vyas, P.R., Patel, R.N., Patel, I.R., Abramson, S.B., 1996. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. Proceedings of the National Academy of Sciences of the United States of America 93, 14014–14019.
Arbabi, M., Bagheri, M., Rezaei, F., Ahmadi-Abhari, S.A., Tabrizi, M., Khaleghi-Sigaroudi, F., Akhondzadeh, S., 2012. A placebo-controlled study of the modafinil added to risperidone in chronic schizophrenia. Psychopharmacology (Berl) 220, 591–598.
Berger, A., 2000. Minocycline slows progress of Huntington's disease in mice. British Medical Journal 321, 70.
Buchanan, R.W., 2007. Persistent negative symptoms in schizophrenia: an overview. Schizophrenia Bulletin 33, 1013–1022.
Chaudhry, I.B., Hallak, J., Husain, N., Minhas, F., Stirling, J., Richardson, P., Dursun, S., Dunn, G., Deakin, B., 2012. Minocycline benefits negative symptoms in early schizophrenia: a randomised double-blind placebo-controlled clinical trial in patients on standard treatment. Journal of Psychopharmacology 26, 1185–1193.
Chaves, C., Marque, C.R., Trzesniak, C., Machado de Sousa, J.P., Zuardi, A.W., Crippa, J.A., Dursun, S.M., Hallak, J.E, 2009. Glutamate-N-methyl-D-aspartate receptor modulation and minocycline for the treatment of patients with schizophrenia: an update. Brazilian Journal of Medical and Biological Research 42, 1002–1014.
Chouinard, G., Margolese, H.C., 2005. Manual for the Extrapyramidal Symptom Rating Scale (ESRS). Schizophrenia Research 76, 247–265.

Denovan-Wright, E.M., Devarajan, S., Dursun, S.M., Robertson, H.A., 2002. Maintained improvement with minocycline of a patient with advanced Huntington's disease. Journal of Psychopharmacology 16, 393–394.

Du, Y., Ma, Z., Lin, S., Dodel, R.C., Gao, F., Bales, K.R., Triarhou, L.C., Chernet, E., Perry, K.W., Nelson, D.L., Luecke, S., Phebus, L.A., Bymaster, F.P., Paul, S.M., 2001. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proceedings of the National Academy of Sciences of the United States of America 98, 14669–14674.

Farokhnia, M., Sabzabadi, M., Pourmahmoud, H., Khodaie-Ardakani, M.R., Hosseini, S.M.R., Yekehtaz, H., Tabrizi, M., Rezaei, F., Salehi, B., Akhondzadeh, A., A double-blind, placebo controlled, randomized trial of riluzole as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia. Psychopharmacology (Berlin) 2013, http://dx.doi.org/10.1007/s00213-013-3261-z.

Fujita, Y., Ishima, T., Kunitachi, S., Hagiwara, H., Zhang, L., Iyo, M., Hashimoto, K., 2008. Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the antibiotic drug minocycline. Progress in Neuropsychopharmacology and Biological Psychiatry 32, 336–339.

Ghaleiha, A., Noorbala, A.A., Farnaghi, F., Hajiazim, M., Akhondzadeh, S., 2010. A double-blind, randomized, and placebo-controlled trial of buspirone added to risperidone in patients with chronic schizophrenia. Journal of Clinical Psychopharmacology 30, 678–682.

Hamilton, M., 1960. A rating scale for depression. Journal of Neurology, Neurosurgery, and Psychiatry 23, 56–62.

Kantrowitz, J.T., Javitt, D.C., 2010. N-methyl-D-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? Brain Research Bulletin 83, 108–121.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia Bulletin 13, 261–276.

Kirkpatrick, B., Fenton, W.S., Carpenter , W.T., Marder, S.R., 2006. The NIMH-MATRICS consensus statement on negative symptoms. Schizophrenia Bulletin 32, 214–219.

Levkovitz, Y., Mendlovich, S., Riwkes, S., Braw, Y., Levkovitch-Verbin, H., Gal, G., Fennig, S., Treves, I., Kron, S., 2010. A double-blind, randomized study of minocycline for the treatment of negative and cognitive symptoms in early-phase schizophrenia. Journal of Clinical Psychiatry 71, 138–149.

Leucht, S., Corves, C., Arbter, D., Engel, R.R., Li, C., Davis, J.M., 2009. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 373, 31–41.

Macdonald, H., Kelly, R.G., Allen, E.S., Noble, J.F., Kanegis, L.A., 1973. Pharmacokinetic studies on minocycline in man. Clinical Pharmacology and Therapeutics 14, 852–861.

Miyaoka, T., Yasukawa, R., Yasuda, H., Hayashida, M., Inagaki, T., Horiguchi, J., 2008. Minocycline as adjunctive therapy for schizophrenia: an open-label study. Clinical Neuropharmacology 31, 287–292.

Murphy, B.P., Chung, Y.C., Park, T.W., McGorry, P.D., 2006. Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. Schizophrenia Research 88, 5–25.

National Institute for Clinical Excellence, March 2009. Core interventions in the treatment and management of schizophrenia in primary and secondary care (update), London.

Pi, R., Li, W., Lee, N.T., Chan, H.H., Pu, Y., Chan, L.N., Sucher, N.J., Chang, D.C., Li, M., Han, Y., 2004. Minocycline prevents glutamate-induced apoptosis of cerebellar granule neurons by differential regulation of p38 and Akt pathways. Journal of Neurochemistry 91, 1219–1230.

Rezaei, F., Mohammad-Karimi, M., Seddighi, S., Modabbernia, A., Ashrafi, M., Salehi, B., Hammidi, S., Motasami, H., Hajiaghaee, R., Tabrizi, M., Akhondzadeh, S., 2013. Memantine add-on to risperidone for treatment of negative symptoms in patients with stable schizophrenia: randomized, double-blind, placebo-controlled study. Journal of Clinical Psychopharmacology 33, 336–342.

Tsai, G., Lane, H.Y., Yang, P., Chong, M.Y., Lange, N., 2004. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biological Psychiatry 55, 452–456.

Tuominen, H.J., Tiihonen, J., Wahlbeck, K., 2005. Glutamatergic drugs for schizophrenia: a systematic review and meta-analysis. Schizophrenia Bulletin 72, 225–234.

Wang, J., Wei, Q., Wang, C.Y., Hill, W.D., Hess, D.C., Dong, Z., 2004. Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. Journal of Biological Chemistry 279, 19948–19954.

Wang, X., Zhu, S., Drozda, M., Zhang, W., Stavrovskaya, I.G., Cattaneo, E., Ferrante, R. J., Kristal, B.S., Friedlander, R.M., 2003. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proceedings of the National Academy of Sciences of the United States of America 100, 10483–10487.

Yrjanheikki, J., Tikka, T., Keinanen, R., Goldsteins, G., Chan, P.H., Koistinaho, J., 1999. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proceedings of the National Academy of Sciences of the United States of America 96, 13496–13500.

Zhang, L., Shirayama, Y., Iyo, M., Hashimoto, K., 2007. Minocycline attenuates hyperlocomotion and prepulse inhibition deficits in mice after administration of the NMDA receptor antagonist dizocilpine. Neuropsychopharmacology 32, 2004–2010.

Zhang, W., Narayanan, M., Friedlander, R.M., 2003. Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. Annals of Neurology 53, 267–270.