Schizophrenia Research xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

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

Changes in plasma levels of nitric oxide metabolites and negative symptoms after 16-week minocycline treatment in patients with schizophrenia☆

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ARTICLE INFO

Article history:
Received 11 October 2017
Received in revised form 11 February 2018
Accepted 3 March 2018
Available online xxxx

Keywords:
Minocycline
Nitric oxide
Schizophrenia
Psychopathology
Negative symptoms

ABSTRACT

Objective: This study examined the effect of adjunctive minocycline on psychopathology and possibly relevant biomarkers in patients with schizophrenia.
Method: In a 16-week randomized, double-blind, placebo-controlled study, subjects received either minocycline (200 mg per day) or placebo. Psychopathology was assessed using the Scale for the Assessment of Negative Symptoms (SANS) and the Positive and Negative Syndrome Scale (PANSS) at baseline and week 16. Plasma levels of tumor necrosis factor α (TNFα), interleukin-1 β (IL-1β) and nitric oxide metabolites were assessed at both time points.
Results: Fifty-five patients completed the study (27 in the minocycline group, 28 in the placebo group). The minocycline group had significant decreases in the SANS total sore, the PANSS total score and the PANSS negative symptoms score at week 16 compared to the placebo group. In addition, the minocycline group had a significant decrease in plasma levels of nitric oxide metabolites, but no significant difference in changes in plasma levels of IL-1β or TNF-α, compared to the placebo group at week 16. Further, the more decrease in plasma levels of nitric oxide metabolites was associated with less improvement in negative symptoms.
Conclusion: The beneficial effect of adjunctive minocycline treatment on negative symptoms might be through mechanisms other than the nitric oxide pathway. The implications for future studies were discussed.

1. Introduction

Schizophrenia is a severe, chronic and debilitating mental illness. Although currently available antipsychotic medications are effective in reducing positive symptoms, these agents have only limited effect in mitigating negative symptoms and cognitive deficits, which are associated with functional disability in this patient population (Harvey, 2014). Enormous effort has been invested to explore novel treatment strategies for these two important symptom domains but little progress has been made so far.

Minocycline is a semi-synthetic second-generation tetracycline with antimicrobial and anti-inflammatory effects. It has a high oral bioavailability, excellent penetration to the brain, and is well tolerated in humans (Oya et al., 2014; Zhang and Zhao, 2014). Recent clinical studies including a report from our own group have suggested that minocycline may help improve schizophrenia symptoms, in particular negative symptoms (Chaudhry et al., 2012; Ghanizadeh et al., 2014; Kelly et al., 2015; Khodaie-Ardakani et al., 2014; Liu et al., 2014). However, the mechanisms by which minocycline exerts its beneficial effect in schizophrenia remain unclear.

Growing evidence has suggested that immune activation, cytokine imbalance and an upregulated inflammatory status associated with activated microglia may play a key role in schizophrenia psychopathology (Kirkpatrick and Miller, 2013; Monji et al., 2013; Potvin et al., 2008). Previous research has tried to identify specific inflammatory markers

☆ The Clinical trials.gov identifier: NCT01493622.

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https://doi.org/10.1016/j.schres.2018.03.003
0920-9964/

in relation to schizophrenia. For example, both Naudin et al. (Naudin et al., 1997) and Lin et al. (Lin et al., 1998) found that, compared with healthy controls, patients with chronic schizophrenia had significantly higher serum levels of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6). Our group reported that serum levels of interleukin-1β (IL-1β), IL-6, TNF-α were significantly elevated in patients with drug naïve, first episode schizophrenia compared with healthy controls (Song et al., 2014). Given minocycline's anti-inflammatory property, it has been speculated that minocycline may improve schizophrenia symptoms through suppression of microglia-mediated inflammation (Garrido-Mesa et al., 2013; Pang et al., 2012).

An accumulating body of literature has suggested that nitric oxide (NO) and its metabolites (nitrites and nitrates) may play an important role in schizophrenia (Bernstein et al., 2005; Nasyrova et al., 2015). Low levels of nitric oxide metabolites in the cerebrospinal fluid have been found in patients with schizophrenia (Ramirez et al., 2004). More recently, studies have reported that sodium nitroprusside, a donor of nitric oxide, significantly improved psychopathology and cognitive deficits in patients with schizophrenia (Hallak et al., 2013; Maia-de-Oliveira et al., 2015).

Studies have shown that tetracyclines including minocycline inhibit the expression of NO synthase (Amin et al., 1996; Amin et al., 1997). In a mouse animal model of depression, it was found that minocycline attenuated depressive-like behavior and decreased hippocampal nitrite level (Saeedi Saravi et al., 2016). However, it is unclear whether minocycline might affect NO and its metabolites in patients with schizophrenia, and if yes, whether NO might play a role in moderating or mediating therapeutic response of minocycline in treating schizophrenia.

We previously reported that 16-week adjunctive minocycline significantly improved negative symptoms in risperidone treated patients with early schizophrenia (Liu et al., 2014). Based on the same study, the aims of the present report were to: 1) examine the effect of 16-week minocycline on plasma levels of nitric oxide metabolites and inflammatory markers including IL-1β and TNFα; 2) examine the potential moderating role of these biomarkers in clinical symptom changes after 16-week treatment.

2. Methods

2.1. Participants

Adult patients with schizophrenia were recruited from the 1st Affiliated Hospital of Kunming Medical University, and the Mental Health Center of Yunnan Province, China, between June 2010 and November 2011. The diagnosis of schizophrenia was made by trained clinical interviewers using the structured clinical interview for DSM-IV diagnosis (SCID) (First et al., 1996). Other inclusion criteria included: (1) age 18 to 40 years old; (2) disease duration ≤5 years; (3) on stable dose of risperidone for at least 4 weeks prior to screening; (4) a stable living arrangement. Exclusion criteria were: (1) being allergic to minocycline or tetracycline; (2) a psychiatric diagnosis other than schizophrenia (determined by the SCID); (3) serious medical conditions including unstable heart disease, uncontrollable hypertension, end-stage cancer, liver or kidney disease; (4) diagnosis of diabetes mellitus; (4) female patients who were planning to become pregnant, or were pregnant or lactating. The study was approved by the ethics committee of the 1st Affiliated Hospital of Kunming Medical University, and registered at https://www.clinical trials.gov (identifier: NCT01493622).

2.2. Procedures

After signing the written informed consent, potential subjects went through the screening procedure. Then each eligible subject underwent baseline assessments including the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982), the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), and blood draw for lab tests. Subjects continued to take risperidone at the same dose throughout the study, and were randomized in a 1:1 ratio to receive either minocycline (200 mg per day) or placebo for 16 weeks. At week 16, baseline assessments were repeated.

2.3. Measures

Anthropometric measures included weight, height and body mass index (BMI). Blood samples were obtained 11 h (±1 h) after the completion of the last meal the night before. Subjects were allowed to drink water during the fasting period. Laboratory assays for plasma levels of TNFα, and IL-1β were performed using the enzyme-linked immunosorbent assay (ELISA) (Bender Med Systems GmbH Campus Vienna Biocenter 2 A-1030 Vienna, Austria, Europe). Plasma levels of NO metabolites (including nitrites and nitrates) were measured using the Griess method (Cortas and Wakid, 1990) (Nanjing Jiancheng Bioengineering Institute, China).

2.4. Statistical analysis

Statistical analysis was performed using SPSS (version 24.0, IBM Corp, Armonk, NY). Descriptive statistics were performed to summarize demographic and clinical characteristics of the study sample. Group comparisons were performed using the independent *t*-test for continuous variables, and the Fisher exact test or Chi-square test for categorical variables. Analysis of covariance (ANCOVA) was used to compare change scores from baseline to week 16 between the two treatment groups controlling for baseline scores and potential confounding variables. For all analyses, a p value <0.05 (2-tailed) was used for statistical significance.

3. Results

A total of 79 schizophrenia patients were screened, and 63 were enrolled in this study. Fifty-five patients completed week-16 assessments. Among these 55 completers, 27 were in the minocycline group, 28 in the placebo group. The minocycline group had a significantly shorter disease duration than the placebo group (Mean ± SD, 19.0 ± 12.3 versus 30.2 ± 14.5 months, $p = 0.003$). There were no significant differences between the two groups in age, gender, education level, family history of mental illness, BMI and the daily dosage of risperidone received (p's > 0.2, Table 1).

After controlling for baseline values and disease duration, the ANCOVA analysis showed that the minocycline group had significant decreases, compared to the placebo group, in the SANS total sore (−31.2 ± 25.5 versus −25.3 ± 12.8, $p < 0.001$), the PANSS total score

Table 1
Baseline demographic and clinical characteristics of the study sample.

Variable	Minocycline ($N = 27$)		Placebo ($N = 28$)		p
	Mean	SD	Mean	SD	
Age (years)	26.7	5.5	28.9	7.0	0.220
Education (years)	10.6	3.4	10.6	3.2	0.955
Disease duration (months)	19.0	12.3	30.2	14.5	0.003
Risperidone dosage (mg/day)	3.80	0.90	3.71	0.92	0.741
Variable	**Minocycline ($N = 27$)**		**Placebo ($N = 28$)**		**p**
	N	%	N	%	
Gender					0.874
Male	16	59.3	16	57.1	
Female	11	40.7	12	42.9	
Family history of mental illness					0.446
Yes	6	22.2	4	14.3	
No	21	77.8	24	85.7	

Please cite this article as: Liu, F., et al., Changes in plasma levels of nitric oxide metabolites and negative symptoms after 16-week minocycline treatment in patients with sc..., Schizophr. Res. (2018), https://doi.org/10.1016/j.schres.2018.03.003