A Double-Blind, Randomized Study of Minocycline for the Treatment of Negative and Cognitive Symptoms in Early-Phase Schizophrenia

Yechiel Levkovitz, MD; Shlomo Mendlovich, MD; Sharon Riwkes; Yoram Braw, PhD; Hana Levkovitch-Verbin, MD; Gilad Gal, PhD; Shmuel Fennig, MD; Ilan Treves, MD; and Shmuel Kron, MD

Background: Current antipsychotics have only a limited effect on 2 core aspects of schizophrenia: negative symptoms and cognitive deficits. Minocycline is a second-generation tetracycline that has a beneficial effect in various neurologic disorders. Recent findings in animal models and human case reports suggest its potential for the treatment of schizophrenia. These findings may be linked to the effect of minocycline on the glutamatergic system, through inhibition of nitric oxide synthase and blocking of nitric oxide–induced neurotoxicity. Other proposed mechanisms of action include effects of minocycline on the dopaminergic system and its inhibition of microglial activation.

Objective: To examine the efficacy of minocycline as an add-on treatment for alleviating negative and cognitive symptoms in early-phase schizophrenia.

Method: A longitudinal double-blind, randomized, placebo-controlled design was used, and patients were followed for 6 months from August 2003 to March 2007. Seventy early-phase schizophrenia patients (according to *DSM-IV*) were recruited and 54 were randomly allocated in a 2:1 ratio to minocycline 200 mg/d. All patients had been initiated on treatment with an atypical antipsychotic ≤ 14 days prior to study entry (risperidone, olanzapine, quetiapine, or clozapine; 200–600 mg/d chlorpromazine-equivalent doses). Clinical, cognitive, and functional assessments were conducted, with the Scale for the Assessment of Negative Symptoms (SANS) as the primary outcome measure.

Results: Minocycline was well tolerated, with few adverse events. It showed a beneficial effect on negative symptoms and general outcome (evident in SANS, Clinical Global Impressions scale). A similar pattern was found for cognitive functioning, mainly in executive functions (working memory, cognitive shifting, and cognitive planning).

Conclusions: Minocycline treatment was associated with improvement in negative symptoms and executive functioning, both related to frontal-lobe activity. Overall, the findings support the beneficial effect of minocycline add-on therapy in early-phase schizophrenia.

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Corresponding author: *Yechiel Levkovitz, MD, The Emotion-Cognition Research Center, The Shalvata Mental Health Care Center, POB 94. Hod-Hasharon, Israel (ylevk@clalit.org.il).*

Minocycline is a second-generation tetracycline that exerts anti-inflammatory and antimicrobial effects while having a distinct neuroprotective profile.[1] It has excellent brain tissue penetration, is well tolerated, and is almost completely absorbed when taken orally. These properties, as well as its beneficial effect in animal models of neurologic disorders, led investigators to suggest its potential in the treatment of schizophrenia.[1,2]

The therapeutic potential of minocycline was demonstrated in recent studies using animal models of schizophrenia. The "glutamate hypothesis" links schizophrenia to a dysfunction in glutamatergic neurotransmission via *N*-methyl-D-aspartic acid (NMDA) receptors.[3] This is evident in the fact that administration of NMDA receptor antagonists produces positive/negative symptoms and cognitive impairments in healthy humans, while exacerbating symptoms in schizophrenia patients.[4,5] When using animal models based on the glutamate hypothesis, researchers found minocycline effective. For example, minocycline countered the disruptive effects of an NMDA antagonist on visuospatial memory and sensorimotor gating.[6] Similarly, minocycline attenuated behavioral changes (eg, hyperlocomotion and prepulse inhibition deficits) and the increase of dopamine in the frontal cortex and striatum after administration of MK801 (an NMDA antagonist).[7] In another study, minocycline was able to reduce cognitive disturbances induced by a different NMDA receptor antagonist (phencyclidine).[8]

These preliminary findings in animal models sparked interest in minocycline's potential for the aid of human schizophrenia patients. At this point, 2 preliminary studies on schizophrenia patients were conducted. First, Miyaoka et al[9] reported 2 published case reports of successful treatment of acute catatonic schizophrenia using minocycline (150 mg/d for 2 weeks, discontinued for 1 week and resumed

afterward) added to antipsychotic treatment (haloperidol or haloperidol and risperidone).[9] The researchers concluded that after minocycline treatment the patients were practically symptom free and that minocycline appears to be safe for use in patients with advanced schizophrenia. The effects of minocycline were hypothesized to be related to its action on the NMDA neurotransmitter system.[10] Second, Miyaoka et al[11] reported the treatment with minocycline (adjunct to antipsychotic medication) of 22 schizophrenia patients in a 4-week open-label study (150 mg/d). There were no adverse events, and a clinical improvement was evident with the minocycline treatment, which was maintained at follow-up evaluation 4 weeks after the end of minocycline treatment. The research team concludes that, "augmentation with minocycline may prove to be a viable strategy for 'boosting' antipsychotic efficacy and for treating schizophrenia."[11(p287)]

Minocycline may exert its effect through several possible mechanisms of actions. First, the efficacy of minocycline may be related to its effect of the glutamate pathway. Minocycline is a potent blocker of nitric oxide–induced neurotoxicity.[12,13] Glutamate, acting on NMDA receptors, is the principal activation signal for the production of nitric oxide.[14,15] Activation of NMDA receptor leads to a toxic calcium influx that activates numerous enzymes, including neuronal nitric oxide synthase. Nitric oxide is able to further increase the excitotoxicity by enhancing glutamate release from presynaptic neurons and inhibiting glial glutamate transporters.[16–19] Second, minocycline effects dopamine neurotransmission. Schizophrenia is associated with a dysregulation of dopamine functioning in the prefrontal cortex and striatum.[20] As indicated earlier, pretreatment with minocycline attenuated the increase of dopamine levels in the frontal cortex and striatum following administration of an NMDA antagonist.[7] Minocycline also ameliorates the neurotoxicity caused by methamphetamine,[7,21] with a preliminary finding indicating its ability to attenuate the reduction of dopamine transporters resulting from methamphetamine treatment.[22] Third, Miyaoka et al[11] suggest neurodegeneration as a possible focus for future research; apoptotic cell death is related to microglial activation and the neurotoxic products generated by persistently activated microglia (such as nitric oxide).[23,24] Since minocycline is a potent inhibitor of microglial activation,[21,25] this may prove to be an important line for future research to follow. This mechanism of action deserves special consideration with regard to the current study, in light of evidence for the presence of microglial activation during the first 5 years of schizophrenia onset (the time frame of the current study; elaborated later).[26]

The current study examined the efficacy of minocycline as an add-on therapy for the treatment of schizophrenia. The study focuses on 2 fundamental and related features of schizophrenia[27,28]: (1) negative symptoms, which are core components of schizophrenia[27,29] and share many characteristics with the cognitive symptoms of schizophrenia and (2) executive functions, which are involved in monitoring and regulating lower cognitive processes and in goal-oriented behaviors, such as planning, working memory, and problem solving.[30] These cognitive functions are impaired in disorders that involve frontal hypofunctioning, such as schizophrenia.[31] Negative and cognitive symptoms are correlated in schizophrenia patients and show similarities in prevalence and course, as well as prognostic and functional significance (although the nature of the relationship is still debated[32]). Moreover, negative symptoms and executive functions are both related to prefrontal functioning (ie, dorsolateral prefrontal cortex and the frontal medial cortex dysfunction).[33] This fact is of importance since several lines of investigation revealed prefrontal alterations in schizophrenia. For example, volume reductions were found in the prefrontal cortex of patients with schizophrenia,[34,35] and functional neuroimaging indicated hypofrontality to be a characteristic of schizophrenia.[36,37] This has a clear significance since the frontal lobes have a key role in integrating the products of the other lobes, in emotional regulation and high cognitive functions.[38,39]

The current study is a double-blind, placebo-controlled, randomized study of the effects of minocycline of executive functions and negative symptoms in schizophrenia. These symptoms are only partially ameliorated by existing antipsychotic medications, a fact that has led to an ongoing effort by researchers to develop newer drug treatments specifically aimed at alleviating these symptoms.[40,41] To the best of our knowledge, this is the first time that a more comprehensive assessment of the efficacy and safety of minocycline for the treatment of schizophrenia symptoms has been conducted. Such an investigation is needed in light of the limited effect of existing antipsychotics and the promise that minocycline holds.

We hypothesized that the effect of minocycline add-on therapy would surpass that of treatment with atypical antipsychotics alone. We focused on patients in the early stage of schizophrenia in light of findings indicating that early pharmacologic intervention could improve the course of the disorder.[42] In addition, we aimed to reduce the deterioration that occurs primarily in the early stages of the disorder (generally confined to the first 5 years after onset[43]). Choosing an early phase also promised a relatively short exposure of patients to prior antipsychotics. A follow-up of 6 months allowed enough time for the effect of minocycline on negative symptoms and cognitive deficits to become evident. The design of the study is in good fit with many of the points raised by the 2 consensus statements on negative symptoms and cognitive symptoms.[44,45] However, the specific aims of the study lead to several deviations. First, the study focuses on patients at the first years after onset and therefore does not include clinically stable patients. Second, the consensus statements on cognitive symptoms (question 2) also call for the inclusion of subjects with no more than a "moderate"

Table 1. Patient Accountability by Visit During a 6-Month Study of Minocycline Add-On Treatment Versus Placebo in Early-Phase Schizophrenia

	Lead-In Phase		Study Phase							
Group	Visit 1, Week −2	Visit 2, Week −1	Visit 3, Week 0	Visit 4, Week 1	Visit 5, Week 2	Visit 6, Week 6	Visit 7, Week 10	Visit 8, Week 14	Visit 9, Week 18	Visit 10, Week 22
Experiment, n[a]	70		36	33	33	23	21	16	14	13
Control, n[b]			18	17	17	11	11	9	8	8
Total, n	70		54	50	50	34	32	25	22	21

[a]Minocycline plus antipsychotics treatment group.
[b]Placebo plus antipsychotics treatment group.

severity of negative symptoms, which cannot be accommodated in the current study (since it focuses on subjects with high rating of negative symptoms).

METHOD

Participants

Potential candidates from the Shalvata Mental Health Center and Abarbanel Medical Mental Health Center (both affiliated with Tel-Aviv University) underwent screening (n = 137). The inclusion criteria were subjects who (1) were aged 18–35 years; (2) were currently diagnosed with *DSM-IV* schizophrenia, confirmed by the Structured Clinical Interview for *DSM-IV* (SCID),[46] conducted by a trained psychiatrist; (3) were in an early phase of the disorder (ie, within 5 years of their first exposure to neuroleptic treatment[47]); (4) did not receive antipsychotic treatment for 6 months preceding current symptom exacerbation; (5) had a baseline total score > 60 on the Positive and Negative Syndrome Scale (PANSS)[48]; (6) had been initiated on treatment with atypical antipsychotic medication ≤ 14 days prior to study entry (risperidone, olanzapine, quetiapine, or clozapine; 200–600 mg/d chlorpromazine-equivalent doses); and (7) were able to comprehend the procedure and aims of the study. Exclusion criteria were subjects who (1) had acute, unstable, significant, or untreated medical illness besides schizophrenia; (2) were pregnant or breastfeeding; (3) had a *DSM-IV* diagnosis of substance abuse or dependency; (4) were taking a known contraindication to minocycline treatment; (5) had received treatment with minocycline or β-lactam antibiotics in the preceding half year before study entry; and (6) were under compulsory hospitalization. The study was conducted from August 2003 to March 2007.

Enrolled patients were dropped from the study in case of (1) serious adverse effects from medications, (2) required changes in the dose or type of antipsychotic during the study, or (3) substantial clinical improvement during the placebo lead-in phase evident in a decrease of > 25% in PANSS scores. After a complete description of the study was given to the subjects, written informed consent was obtained. The research was approved by the institutional and national review boards committees and is in accordance with the Declaration of Helsinki guidelines.

Procedure

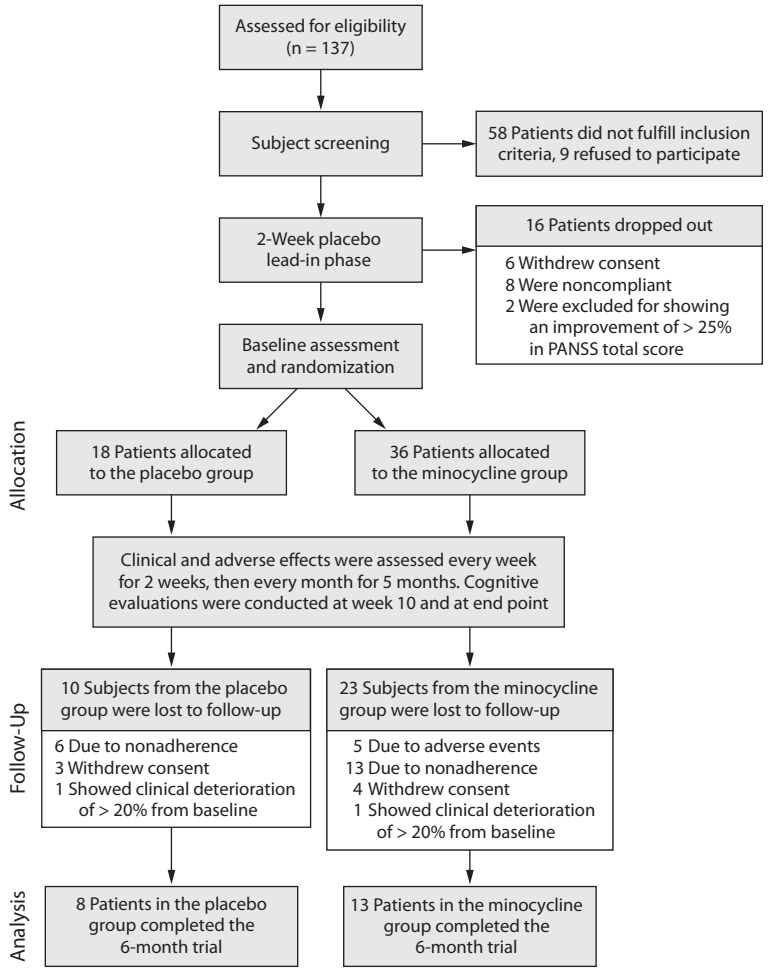
The screening meeting included a description of the study (including potential risks and benefits) and a SCID assessment. Patients who signed the informed consent form and who were eligible according to the inclusion/exclusion criteria entered a 2-week, single-blind, placebo lead-in phase. During this phase, all patients received placebo and were evaluated weekly in order to assess the placebo effect, namely, the improvement of the patients' symptoms unrelated to the study drug. In addition, disallowed concomitant therapy (mood stabilizers, tranquilizers, or anticholinergic medication) was tapered during the lead-in phase. Patients were then randomly assigned to either the minocycline or placebo groups in a 2:1 ratio. They entered the 22-week add-on phase with minocycline or placebo (200 mg/d) being added to their atypical antipsychotic medication. This dose can be considered as the standard human dose of minocycline, supported by the US Food and Drug Administration guidelines.[49] It was found to be safe and well tolerated at 200 mg/d over a 6-month trial duration.[50,51] Dose determination is further considered in the Discussion section. Shor Tabechnik Pharmacy (Israel) provided minocycline or placebo in identical tablet form.

Study duration was 24 weeks including the lead-in phase. Clinical status was evaluated weekly during the lead-in phase and the first 2 weeks of the study (0, week 1, week 2). Clinical status was then evaluated once a month for the remaining 5 months (weeks 6, 10, 14, 18, 22). Cognitive abilities were assessed upon enrollment (week −2) and at weeks 10 and 22 (the Stockings of Cambridge [SOC] task[52] was administered only at weeks −2 and 22). Each participant's weight was measured at study entrance and at study closure. Extrapyramidal side effects were rated at weeks −2, 0, 2, 6, 14, and 22 by the Extrapyramidal Symptoms Rating Scale (ESRS).[53] Diagnosis was reestablished at the end of the study with a follow-up SCID evaluation by an independent psychiatrist. All subjects underwent baseline physical examinations including electrocardiograms. The enrollment, randomization, and follow-up of the study patients are depicted in Table 1 (patient accountability) and the CONSORT diagram (Figure 1).

Outcome Measures

Clinical measures. The Scale for the Assessment of Negative Symptoms (SANS) assesses affective flattening

Figure 1. CONSORT Diagram of Study Course



Abbreviation: PANSS = Positive and Negative Syndrome Scale.

or blunting, alogia, avolition-apathy, anhedonia-asociality, and disturbance of attention[54]; secondary clinical outcome measures consisted of the PANSS, the Clinical Global Impressions scale (CGI),[55] the Calgary Depression Scale for Schizophrenia (CDS),[56] and Insight and Treatment Attitudes Questionnaire (ITAQ).[57]

Cognitive measures. The cognitive evaluation was performed using the Cambridge Neuropsychological Test Automated Battery (CANTAB [Cambridge Cognition Ltd., Cambridge, England]). The CANTAB is comprised of a touch screen computer allowing a rapid and noninvasive assessment of cognitive functions. The following tests were presented in a semirandomized manner:

1. Psychomotor speed. This function was assessed by using the Motor task.[52] The selected measure was response latency (in milliseconds).

2. Attention. This function was measured by using the Rapid Visual Processing (RVP) task.[52] The RVP is a Continuous Performance Test[52] of sustained attention, highly sensitive to brain damage or dysfunction. The selected measure was A', representing the subjects' ability to detect the target sequence regardless of response tendency.

3. Visuospatial memory. Pattern and spatial memory domains were investigated by using the Pattern Recognition Memory (PRM)[52] and Spatial Recognition Memory (SRM).[52] In both tasks, the selected measure was the percentage of correct responses.

4. Executive function. The current study conceptualizes executive functions as a number of different higher-order cognitive processes, allowing dissociations between components of executive functioning to be exploited.[58,59] Such an approach is in line with critiques on the narrow definition of executive functions used in many studies.[60,61] Working memory was tested using the Spatial Working Memory (SWM) task.[52] This task assesses the ability to retain and manipulate information in spatial working memory (selected measure was the number of errors conducted by the subject). Cognitive shifting and flexibility was tested using the IntraDimensional/Extradimensional Set-Shifting (ID/ED) task.[52] The task assesses the ability to shift between intradimensional and extradimensional sets as well as the capacity for reversal learning (scored by the number of completed stages and the number of errors performed before and after the extradimensional shift, pre-extradimensional errors and post-extradimensional errors). Cognitive planning was tested using the CANTAB's version of the Tower of London task (Stockings of Cambridge [SOC]). The task assesses planning and organizing a goal-oriented sequence of actions (scored using the number of problems solved in minimum moves).

Functional measures. The assessment was conducted using the Global Assessment of Functioning Scale (GAF),[62] rating social, occupational, and psychological functioning of patients; Social and Occupational Functioning Assessment Scale (SOFAS),[63] rating social and occupational functioning of patients; and the Multinomah Community Ability Scale (MCAS),[64] assessing impairments and abilities among individuals with severe mental illness living in the community (interpersonal relations and daily living skills subscales were utilized and combined for a total score). Lower scores on the MCAS indicate lower levels of functioning.

Table 2. Baseline Demographic and Disorder-Related Data for the Minocycline and Placebo Groups (total study population and patients completing the study)[a]

	Total Study Population			Study Completers		
Measure	Minocycline	Placebo	P^b	Minocycline	Placebo	P^b
Patients, n	36	18	NA	13	8	NA
Age, mean (SD), y	25.14 (4.77)	24.67 (4.24)	.723[c]	24.8 (4.01)	25.5 (4.06)	.83[c]
Gender, n (%)			.272[d]			.57[d]
Male	25 (69.44)	15 (83.33)	NA	10 (76.92)	7 (87.50)	NA
Female	11 (30.55)	3 (16.66)	NA	3 (23.07)	1 (12.50)	NA
Education level, mean (SD), y	11.97 (0.77)	11.78 (1.06)	.446[c]	11.92 (0.27)	12.12 (0.99)	.49[c]
Living arrangements, n (%)						
Parents	29 (77.77)	12 (66.66)	.944[d]	10 (76.92)	5 (62.5)	.477[d]
Grandparents	1 (2.77)	1 (5.55)	NA	0	1 (12.5)	NA
Spouse	2 (5.55)	1 (5.55)	NA	1 (7.68)	1 (12.5)	NA
Roommates	2 (5.55)	1 (5.55)	NA	1 (7.68)	0	NA
Alone	1 (2.77)	1 (5.55)	NA	1 (7.68)	0	NA
Other	2 (5.55)	2 (11.11)	NA	0	1 (12.5)	NA
Psychiatric history, mean (SD)			NA			NA
Age at first episode, y	21.36 (4.34)	20.94 (4.54)	.744[c]	21.07 (3.45)	22.62 (4.97)	.41[c]
Age at first hospitalization, y	22.44 (4.15)	22.18 (5.05)	.842[c]	22.83 (3.54)	23.86 (5.39)	.62[c]
Hospitalizations, n	1.94 (1.58)	1.56 (0.92)	.341[c]	2.23 (2.20)	1.37 (1.18)	.33[c]
Patients with antipsychotic medication, n (%)			.071[d]			.21[d]
Olanzapine	16 (45.71)	5 (27.77)	NA	4 (30.77)	3 (37.5)	NA
Quetiapine	2 (5.71)	1 (5.55)	NA	0	0	NA
Risperidone	9 (25.71)	11 (61.11)	NA	4 (30.77)	3 (37.5)	NA
Clozapine	8 (22.85)	1 (5.55)	NA	4 (30.77)	1 (12.5)	NA
Patients with a general medical illness, n (%)	7 (19.4)	1 (5.55)	.17[d]	3 (23.07)	1 (12.5)	.549[d]
Patients with psychiatric family history, n (%)			.434[d]			.252[d]
Yes	19 (52.77)	8 (44.44)	NA	8 (61.54)	2 (25.00)	NA
No	16 (44.44)	8 (44.44)	NA	5 (38.46)	4 (50.00)	NA
Unknown	1 (2.77)	2 (11.11)	NA	0	2 (25.00)	NA
Suicide attempts, n (%)	3 (8.33)	1 (5.55)	.713[d]	2 (15.37)	1 (12.5)	.86[d]
Baseline weight, mean (SD), kg	76.52 (13.03)	80.28 (19.67)	.640[c]	76.54 (12.36)	80.64 (19.47)	.59[c]

[a]Minocycline = minocycline + antipsychotics; placebo = placebo + antipsychotics.
[b]All reported *P* values were nonsignificant.
[c]*t* Test.
[d]χ^2.
Abbreviation: NA = not applicable.

Primary and secondary outcome measures. The primary outcome measure was the SANS, assessing negative symptoms in schizophrenia. All other measures were considered secondary:

1. Secondary clinical outcome measures—monitoring health through the assessment of adverse events and serious adverse events during the study by a trained physician.

2. Secondary cognitive outcome measures—analyses were conducted on a composite score of patients' executive functioning ("executive functioning composite score"). The composite score consists of the mean Z score of each participant in CANTAB tasks of working memory (number of errors on the SWM task), cognitive shifting and flexibility (number of stages completed on the ID/ED task), and cognitive planning (number of problems solved in minimum moves on the SOC task).* Separate analyses of cognitive functioning were conducted in other cognitive domains (eg, visuospatial memory), as well as subcomponents of executive functions.

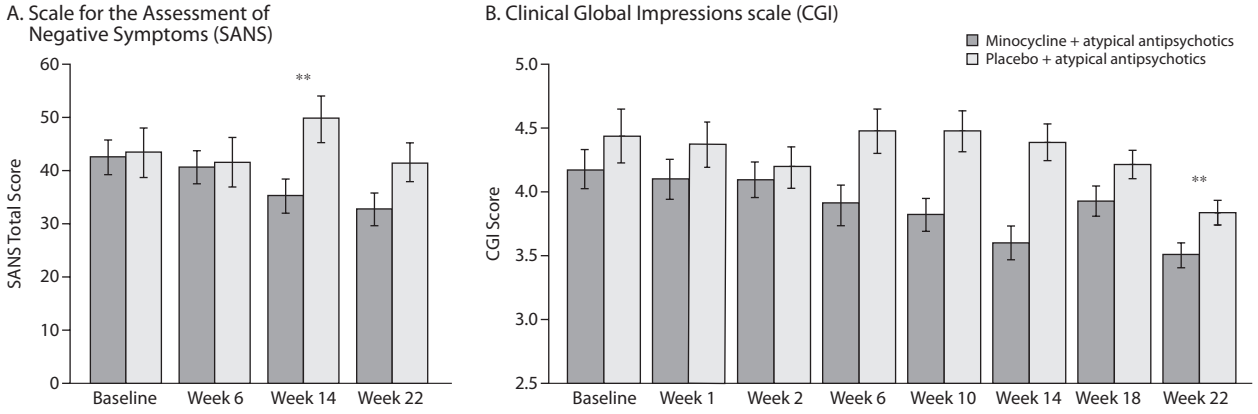
3. Secondary functional outcome measures—all measures listed earlier in the Outcome Measures section).

Data Analysis

General. The current study used the intent-to-treat (ITT) analysis model (analysis was based on the ITT, not on the treatment eventually administered). Intent-to-treat analyses are done to avoid the effects of crossover and dropout, which may break the randomization to the treatment groups in a study.[65] The analysis population consisted of (1) per protocol population—patients who completed the study according to the protocol (ie, completed at least 3 weeks of the study protocol; n = 54), (2) completers—all patients who completed the study according to study protocol, and (3) dropouts—noncompleters in the ITT population. Data for patients who dropped out from the study were imputed using the maximum-likelihood method (expectation maximization algorithm).[66] The use of last observation carried forward (LOCF) was avoided since it makes unwarranted assumptions that may result in either underestimating or overestimating the treatment effects.[67] With regard to psychiatric data, strong time trends are often evident and can be easily confounded with treatment effects.[68] In contrast, the maximum likelihood method appears to produce unbiased

*Note also that (1) the SOC task was assessed only twice (entry and end of study) and therefore was not included in the calculation of Z scores for the assessment at study's midpoint and (2) the SWM measure is reversed (higher number indicating worse performance) and therefore its Z score was reversed.

Figure 2. Mean ± SEM Scores of Clinical Measures Across 22 Weeks for Patients Taking Minocycline Versus Placebo as an Add-On Treatment for Schizophrenia



**$P < .01$.
Abbreviation: SEM = standard error of the mean.

estimates of a treatment effect.[69] Overall, these modern methods have fewer limitations and are less restrictive assumptions than required for LOCF.[70,71] All analyses were conducted using the SPSS statistical analysis software (version 12.0; SPSS Inc, Chicago, Illinois).

Differences in baseline demographic and disorder-related measures were assessed using χ^2 or independent-samples *t* tests (for parametric and nonparametric measures, respectively). These comparisons were made between the 2 treatment groups (overall comparisons and comparisons of completers; Table 2). Subjects' weight change (between study entry and end point) was analyzed using a *t* test (minocycline/placebo groups). Group differences (placebo/minocycline) in the number of prior antipsychotic medications and antipsychotic treatments during the study were analyzed using a *t* test and a χ^2 test, respectively. Repeated-measures analysis of variance (ANOVA) was conducted with between-subjects factors of treatment (placebo/minocycline) and antipsychotic medication (olanzapine, risperidone, quetiapine, or clozapine) and a within-subjects factor of time. These analyses investigated the possibility that antipsychotic medication may serve as an alternative explanation for the findings. The use of Bonferroni corrections[72] was avoided since it can potentially hide meaningful baseline differences.

Clinical, cognitive, and functional assessment. Measures were analyzed using repeated-measures ANOVAs using a between-subjects factor of treatment (placebo/minocycline) and a within-subjects factor of time. A multivariate ANOVA was used for cognitive domains that consisted of several related subscales (ie, executive functions). Bonferroni corrections were employed in ANOVA analyses of related measures in order to keep the total chance of erroneously reporting a difference $\alpha < .05$[72]: (1) clinical measures—corrected $\alpha = .0125$ for the 4 measures PANSS, CGI, CDS, and ITAQ; (2) cognitive measures—corrected $\alpha = .025$ for the 2 measures PRM and SRM CANTAB tasks; and (3) functional measures—corrected $\alpha = .0125$ for the 3 measures GAF, SOFAS, and MCAS.

RESULTS

Baseline Assessments

There were no group differences in demographic and disorder-related measures (see Table 2). There were no differences between the groups in the number of different antipsychotics used prior to the study or the antipsychotic treatment used during the study (note that the power of the analyses was limited by the small number of patients treated with each type of atypical antipsychotic). There were also no differences in dropout rate between the 2 treatment groups ($\chi^2 = 0.35$, not significant). The ANOVA showed no statistically significant antipsychotic medication effect on the outcome measures. Similarly, there were no statistically significant interactions, suggesting that the antipsychotics neither influenced clinical outcome nor interacted with minocycline. No differences were shown in *t* tests between treatment groups in baseline clinical and cognitive data.

Side Effects

In the minocycline group, 2 patients had indigestion, 2 had pigmentation, and 1 attempted suicide. In 4 of the 5 above-mentioned cases, minocycline treatment was discontinued, and the patients were excluded from the study. In 1 case, in which the subject experienced mild pigmentation, treatment was continued as planned. Minocycline had no impact on extrapyramidal symptoms, as evident in the ESRS. No adverse events occurred in the placebo group.

Primary Outcome Measure (negative symptoms)

There was a significant time effect for SANS total scores (Figure 2A and Table 3), indicating that subjects'

Table 3. Clinical and Functional Assessment Results for the Minocycline and Placebo Groups

	Minocycline		Placebo		Time Effect		Treatment Effect		Time × Treatment Interaction	
Measure	Baseline (mean ± SD)	End Point (mean ± SD)	Baseline (mean ± SD)	End Point (mean ± SD)	*P*	Effect Size (*r*)	*P*	Effect Size (*r*)	*P*	Effect Size (*r*)
SANS	42.54 (18.66)	32.61 (19.59)	43.56 (18.12)	41.56 (17.88)	< .001	.51	.18	.18	< .01	.46
PANSS positive	14.50 (4.90)	10.67 (2.42)	15.33 (4.93)	11.19 (4.69)	< .001	.59	.94	.009	.43	.23
PANSS negative	22.33 (5.32)	17.10 (5.91)	22.72 (5.73)	20.32 (6.53)	< .001	.61	.17	.18	.39	.24
PANSS general psychopathology	43.62 (7.50)	34.02 (7.27)	44.15 (7.36)	32.72 (5.50)	< .001	.80	.94	.008	.81	.13
PANSS total	80.37 (12.77)	67.40 (10.25)	82.86 (13.90)	63.80 (18.09)	< .001	.74	.89	.017	.48	.21
CGI	4.19 (0.87)	3.51 (0.45)	4.45 (0.77)	3.84 (0.80)	< .001	.75	< .01	.38	< .01	.61
CDS	4.71 (3.66)	3.16 (1.94)	6.11 (3.26)	4.59 (4.40)	< .05[a]	.40	.12	.21	.23	.28
ITAQ	14.61 (6.26)	17.33 (4.88)	14.83 (5.64)	13.41 (5.79)	.20	.25	.30	.14	< .05[a]	.37
GAF	45.00 (10.82)	56.90 (9.64)	43.26 (9.76)	51.41 (6.30)	< .001	.63	< .01	.37	.06	.37
SOFAS	42.81 (11.97)	57.72 (9.72)	42.09 (7.81)	51.52 (7.92)	< .001	.66	< .01	.36	< .01	.45
MCAS	27.44 (5.25)	28.24 (5.12)	26.33 (5.65)	26.71 (7.45)	.22	.24	.25	.15	.66	.13
ESRS	2.86 (2.95)	2.20 (1.37)	3.56 (2.58)	2.64 (3.50)	.37	.28	.25	.16	.87	.15

[a]Not significant after Bonferroni correction.
Abbreviations: CDS = Calgary Depression Scale for Schizophrenia, CGI = Clinical Global Impressions scale, ESRS = Extrapyramidal Symptoms Rating Scale, GAF = Global Assessment of Functioning Scale, ITAQ = Insight and Treatment Attitudes Questionnaire, MCAS = Multinomah Community Ability Scale, PANSS = Positive and Negative Syndrome Scale, SANS = Scale for the Assessment of Negative Symptoms, SOFAS = Social and Occupational Functioning Assessment Scale.

Table 4. Cognitive Assessment Results for the Minocycline and Placebo Groups

	Minocycline		Placebo		Time Effect		Treatment Effect		Time × Treatment Interaction	
Measure	Baseline (mean ± SD)	End Point (mean ± SD)	Baseline (mean ± SD)	End Point (mean ± SD)	*P*	Effect Size (*r*)	*P*	Effect Size (*r*)	*P*	Effect Size (*r*)
Executive functions										
Executive functioning composite score	−0.063 (0.851)	0.286 (0.712)	0.126 (0.715)	−0.572 (0.793)	.19	.17	.32	.13	< .001	.47
Psychomotor speed										
Motor task latency	898.52 (301.98)	857.73 (182.63)	928.82 (261.03)	741.94 (108.87)	< .05	.44	.44	.10	NS	.37
Attention										
RVP A'	0.86 (0.05)	0.88 (0.05)	0.89 (0.06)	0.90 (0.05)	< .001	.50	.06	.25	.54	.15
Memory										
SRM, % correct	76.00 (16.47)	74.77 (15.57)	76.66 (13.82)	51.11 (21.69)	< .001	.65	< .05[a]	.29	< .001	.61
PRM, % correct	81.57 (11.39)	89.39 (13.05)	85.26 (8.43)	85.42 (8.90)	.09	.30	.72	.04	.13	.28
Executive functions										
ID/ED pre-extradimensional errors	8.25 (7.12)	7.94 (4.69)	9.05 (6.23)	7.81 (3.05)	.07	.22	.90	.02	.69	.08
ID/ED post-extradimensional errors	15.05 (11.49)	7.83 (7.12)	11.83 (11.35)	19.09 (10.61)	.52	.11	.16	.19	< .001	.42
ID/ED stages completed	7.99 (1.33)	8.47 (0.62)	8.27 (0.89)	7.88 (0.85)	.69	.08	.99	.00	< .01	.31
SOC problems solved	7.34 (2.28)	8.79 (2.03)	7.33 (2.54)	7.31 (1.80)	< .01	.36	.20	.18	< .01	.36
SWM errors	42.45 (23.65)	29.45 (20.74)	35.33 (25.1)	55.46 (20.44)	.26	.23	.20	.18	< .001	.58

[a]Not significant after Bonferroni correction.
Abbreviations: ID/ED = Intradimensional/Extradimensional Set-Shifting, NS = not significant, PRM = Pattern Recognition Memory, RVP = Rapid Visual Processing, SOC = Stockings of Cambridge, SRM = Spatial Recognition Memory, SWM = Spatial Working Memory.

psychopathology lessened with study progression ($F_{3,50} = 5.95$, $P < .001$). There was also a significant time × treatment interaction with SANS total scores ($F_{3,50} = 4.44$, $P < .01$); follow-up analysis indicated that minocycline alleviated negative symptoms (time effect, $P < .01$), with SANS scores continuing to decrease, starting from week 14 ($P < .05$). In contrast, the placebo group deteriorated as indicated by an increase in negative symptoms (a time effect in SANS scores, $P < .05$).

Secondary Outcome Measures

Clinical evaluations. A significant treatment effect was found on the CGI, with lower (better) scores in the minocycline group compared to the placebo group ($F_{1,52} = 8.64$, $P < .01$). A significant time effect was observed on PANSS and CGI, indicating that subjects' psychopathology lessened with study progression ($F_{9,44} = 12.67$, $P < .001$ and $F_{7,46} = 8.38$, $P < .001$, respectively). Finally, a significant time × treatment interaction was found on the CGI ($F_{7,46} = 3.84$, $P < .01$).

Follow-up analysis indicated that minocycline improved clinical status (time effect, $P < .001$), with CGI scores continuing to decrease, starting from week 14 ($P < .01$). In contrast, the placebo group showed no statistically significant change in CGI scores (Figure 2B and Table 3).

Cognitive evaluations.

1. Psychomotor speed. The Motor Task (psychomotor speed) responses showed a time effect, indicating a decrease in response latency with study progression ($F_{1.56,81.61} = 3.85$, $P < .05$) (Table 4).

2. Attention. The RVP task (sustained attention) showed a time effect for the probability to detect a target, with the performance of both groups improving with study progression ($F_{2,51} = 8.67$, $P < .001$). There was no treatment or time × treatment interaction, although the performance of the minocycline group improved throughout the study (time effect, $P < .01$), with no change in the placebo group.

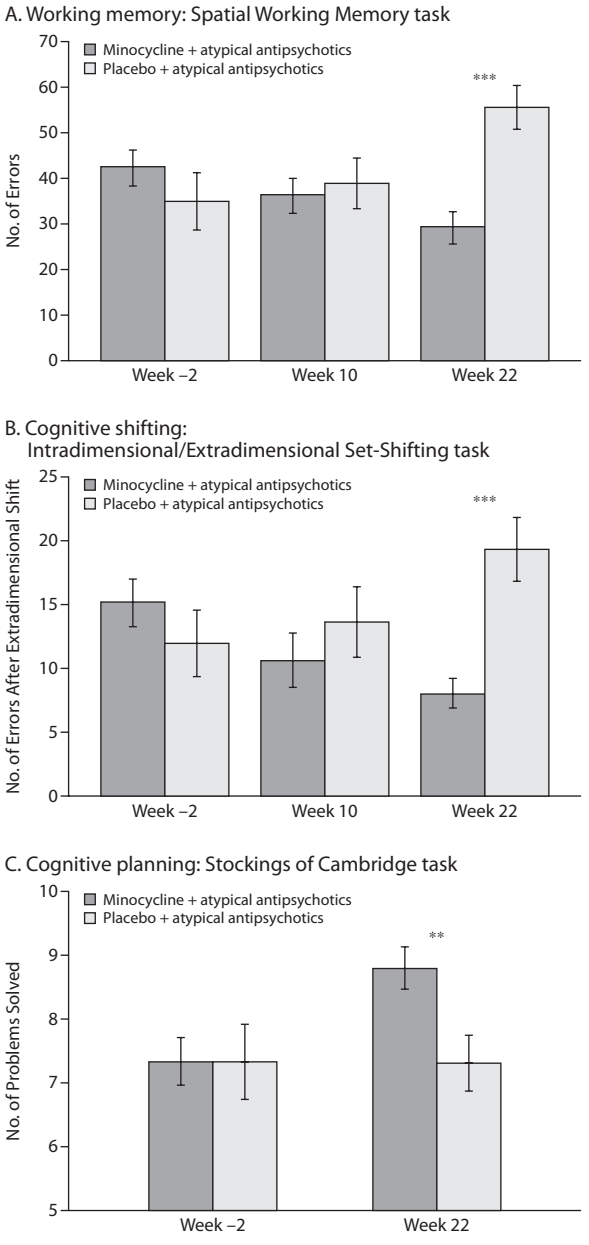
3. Visuospatial memory. In the SRM task, spatial memory showed a significant time main effect and a time × treatment interaction ($F_{2,51} = 18.99$, $P < .001$ and $F_{2,51} = 14.95$, $P < .001$, respectively). Follow-up analyses indicated an initial decline in the minocycline group's performance (time effect, $P < .001$), with an improvement in the last visit ($P < .001$). In contrast, the placebo group deteriorated continuously throughout the study (time effect, $P < .001$). No significant main effects or interactions were found in the PRM task (pattern memory).

4. Executive functioning (composite score). There was a significant time × treatment interaction for the executive functioning composite score ($F_{2,104} = 14.88$, $P < .001$); follow-up paired-samples t tests (for each group separately) indicated that there were differences in the executive functioning composite score between the first and second cognitive assessment. However, significant differences were evident between the second and third assessments; while the minocycline-treated patients improved their executive functioning between the second and third assessment ($t_{35} = -2.31$, $P < .05$), the placebo group deteriorated in their executive functions between the 2 assessments ($t_{17} = 5.31$, $P < .001$).

5. Executive function (subcomponent 1; working memory). The SWM task revealed a time × treatment interaction for the number of errors performed ($F_{2,51} = 12.91$, $P < .001$) (Figure 3). Follow-up analysis showed that, in the minocycline group, the number of errors decreased throughout the study, whereas it increased in the placebo group (time effect, $P < .05$ and $P < .01$, respectively).

6. Executive function (subcomponent 2; cognitive planning). The SOC task showed a time effect accompanied by a time × treatment interaction ($F_{1,52} = 7.59$, $P < .01$ and $F_{1,52} = 7.97$, $P < .01$, respectively); follow-up analysis indicated that the number of problems solved in minimum moves by the minocycline group increased with study progression (time effect, $P < .001$), while no change was evident in the placebo group.

Figure 3. Mean ± SEM Scores of Executive Functions: Working Memory, Cognitive Shifting, And Cognitive Planning



**$P < .01$.
***$P < .001$
Abbreviation: SEM = standard error of the mean.

7. Executive function (subcomponent 3; cognitive shifting). The ID/ED results revealed a time effect accompanied by a time × treatment interaction ($F_{6,47} = 2.57$, $P < .05$ and $F_{6,47} = 5.49$, $P < .001$, respectively). The interaction was significant for the number of completed stages and the post-extradimensional errors ($F_{1.7,88.4} = 5.59$, $P < .01$ and $F_{1.6,88.3} = 10.88$, $P < .001$, respectively); follow-up analysis revealed that the minocycline group improved their ID/ED

performance (decrease in post-extradimensional errors, $P < .01$ and increase in stages completed, $P < .05$), while the placebo group did not.

Functional evaluations. There was an overall functional improvement as indicated by a time effect for the GAF and SOFAS ($F_{3,50} = 10.88$, $P < .001$ and $F_{3,50} = 13.10$, $P < .001$, respectively). A treatment effect was evident in the GAF and SOFAS, with higher (better) scores in the minocycline group ($F_{1,52} = 8.09$, $P < .01$ and $F_{1,52} = 7.94$, $P < .01$, respectively). There was also a time × treatment interaction for the SOFAS ($F_{3,50} = 4.23$, $P < .01$); follow-up analysis showed that the SOFAS scores of both groups increased (improved) with study progression (time effect, $P < .001$ and $P < .01$, respectively). The minocycline group, however, reached higher SOFAS scores than the placebo group in all visits except baseline ($P < .05$; see Table 3).

Weight measurements. At baseline, there were no significant weight differences between patients belonging to the 2 groups (see Table 2). Patients in the placebo group gained significantly more weight during the study compared to the minocycline group (10.7 kg and 2.08 kg, respectively; $P < .05$). One hundred percent of the patients who received atypical antipsychotics and placebo gained weight compared to only 40% of patients who also received minocycline as an adjuvant. In fact, half of the patients in the minocycline group lost weight.

DISCUSSION

The current study assessed the effects of minocycline on negative symptoms and cognitive deficits in schizophrenia. For this aim, a prospective, double-blind, placebo-controlled design was utilized, with patients followed for 6 months. Overall, the findings point toward a beneficial effect of minocycline as an add-on treatment for schizophrenia patients at an early stage of the disorder.

The effects of minocycline on negative symptoms were evident in a reduction of SANS and CGI scores. In contrast, the placebo treatment did not alleviate negative symptoms, with some measures actually deteriorating during the study. Minocycline was also associated with improved cognitive functioning as assessed by the CANTAB computerized assessment battery. A consistent pattern emerged as minocycline was associated with better executive functioning with study progression (working memory, cognitive flexibility and planning). Taken together, the findings imply the involvement of frontal lobe circuits and correspond to earlier findings of progressive frontal lobe volume reductions in schizophrenia.[73–75] The overall findings suggest that minocycline alleviated or even reversed the expected early deterioration of frontal lobe–related functioning.

Minocycline affected the patients' social and occupational functioning as assessed by the SOFAS questionnaire. The functional improvement may stem from minocycline's effect on cognitive and negative symptoms, serving as mediating factors. This suggestion is in line with evidence of an association between negative/cognitive symptoms and social disability. Correspondingly, Kurtz et al[76] advised that, in order to maximize rehabilitation outcome, pharmacologic interventions should specifically focus on cognitive functioning (eg, problem-solving ability). The lack of effect of minocycline on other functional measures could be attributed to the existence of intervening variables (eg, social cognition). These variables mediate cognitive changes and functional outcome, possibly leading to longer latency for psychotherapeutic effects to translate into actual functional improvements.[77] Furthermore, functioning is effected by socioeconomic factors, such as education opportunities, employment, and social support, which are beyond the control of a clinical study.[78] This may have led to a partial dissociation between the functional outcome and biologic changes induced by minocycline.

Patients treated with minocycline gained less weight when compared to placebo-treated patients. This finding is noteworthy and may take part of the ongoing effort to combat weight gain in schizophrenia.[79,80] Antipsychotic treatment is associated with significant body weight gains in up to 80% of patients.[81,82] This body weight gain has repercussions on physical health,[83] affects patients' quality of life, and is associated with poor adherence to therapy.[84] Antipsychotics induce weight gain, mainly through appetite stimulation.[85] This suggests that minocycline effects on weight may be related to the glutamate system, since NMDA receptor antagonism indirectly disrupts normal satiety signals arising from stomach.[86,87] This may hold important therapeutic implications. For example, amantadine decreases appetite by stimulating dopamine effects and blocking NMDA receptors and was effective in assisting body weight control in schizophrenia.[85] Other mechanisms of action can be hypothesized but are at this point more speculative. For example, schizophrenia involves alterations in proinflammatory cytokines (eg, interleukin-6 [IL-6])[88] that play a part in a feedback model for control and regulation on body fat stores.[89] Minocycline dose dependently reduced tumor necrosis factor-α and IL-6 release by microglia.[90] One last general remark: weight gain is a greater problem for young patients experiencing first-episode psychosis.[91,92] Therefore, the effects of minocycline may be more easily noted in this population (similar to that used in the current study). This is an additional issue that warrants further research.

The current study indicates that minocycline (200 mg/d; 6-month duration) is well tolerated and safe for schizophrenia patients. This conclusion is in line with studies in other disorders, for example Huntington's disease.[50] Studies indicate that hyperpigmentation is most likely related to cumulative doses greater than 70 g, takes longer than 6 months to appear, and is not related to age or concomitant medication use.[93] A recent phase 3 study indicated that minocycline led to a more rapid decline in amyotrophic lateral sclerosis (ALS) patients.[94] While still debated,[95,96] it should

be noted that these side effects in ALS *do not* appear to correlate with deterioration[95] and that minocycline 200 mg/d (6-month duration) was found to be safe in patients with ALS in earlier studies.[96,97] This conclusion is strengthened by the fact that a variety of additional human conditions were treated with a 200-mg dose of minocycline and researchers found it to be safe (for example, acute stroke, prurigo pigmentosa, and acne[93,98,99]). Overall, the current study supports the safety of using minocycline 200 mg/d in schizophrenia, adding data to the 2 earlier studies on human schizophrenia patients that used a somewhat lower dose (treated with 150 mg/d for 2 weeks, discontinued for 1 week, and resumed afterward).[9,11] Regrettably, there is a difficulty in cross-species comparisons with regard to dose determination; studies on animal models used minocycline doses that were almost 30 times the weight-based dose routinely administered to humans.[100] This state of affairs has begun to change lately. For example, a 3-mg/kg dose (a rough equivalent to the 200-mg dose used in humans) did not significantly affect hemodynamic and physiologic variables in a rodent model of infarct.[101]

Several limitations of the study should be mentioned before conclusion. First, the small number of patients treated with each antipsychotic drug did not allow a clear assessment of possible interactions between minocycline and antipsychotic treatment. Clozapine, in particular, necessitates careful monitoring, since it is able to interact with glutamatergic mechanisms via actions at the NMDA/glycine receptor.[102,103] A meta-analysis of randomized controlled trials of glutamatergic drugs indicated *no* significant difference in results of trials in which clozapine was or was not used.[104] This issue should be addressed in future studies. Second, our 6-month follow-up period might have been too short to allow for the effect of minocycline becoming fully evident (for example, on patients' functioning). Third, it could be argued that the results may be explained by a low completion rate. Dropout rates in randomized clinical trials of antipsychotics are high and increase with trial length.[105] For example, Rabinowitz and Davidov[106] review placebo-controlled studies and report dropout rates that range from 53.9% to 85.9% for trials lasting longer than 12 weeks.[107–112] The dropout rates in the current study, therefore, are within range of those found in earlier studies. It should also be noted that study patients in both placebo and experimental groups who completed the study had similar demographic and disorder-related data (including medication), limiting the risk of biased dropout in the treatment groups. Fourth, the mechanism of action through which minocycline acts is not fully elucidated and may differ from those suggested in the article. For example, minocycline also effects p38 mitogen-activated protein kinase, caspase-1 and caspase-3 expression, and cytochrome *c* release.[113,114] In this regard, it should also be noted that the current study did not assess smoking and alcohol consumption, which may effect relevant neurotransmitter systems.

Overall, the current study points toward the promise that minocycline holds for the treatment of schizophrenia. Minocycline may prove as a mean to affect the course of the disorder and its most resilient and debilitating symptoms, cognitive functioning, and negative symptoms. The importance of the findings stem from the limitations of current treatments of schizophrenia to ameliorate these symptoms. Our findings will hopefully motivate further research of minocycline as a pharmacologic intervention for treating schizophrenic patients at the initial stages of the disorder. It would be important to replicate the investigation with an even larger subject cohort and for a longer period of time.

Drug names: amantadine (Symmetrel and others), clozapine (FazaClo, Clozaril, and others), haloperidol (Haldol and others), minocycline (Dynacin, Minocin, and others), olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal and others).

Author affiliations: Emotion-Cognition Research Center, Shalvata Mental Health Care Center, Hod-Hasharon (Drs Levkovitz, Mendlovich, Braw, Fennig, Treves, and Kron and Ms Riwkes); Tel-Aviv University, Tel-Aviv (Drs Levkovitz, Mendlovich, Levkovitch-Verbin, Fennig, Treves, and Kron); and School of Behavioral Sciences, Tel-Aviv Jaffa Academic College, Tel-Aviv Jaffa (Dr Gal), Israel.

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