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Adjunctive Minocycline in Clozapine-Treated Patients with Schizophrenia: Analyzing the Effects of Minocycline on Clozapine Plasma Levels

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Keywords

Schizophrenia; Clozapine; Drug interaction; Minocycline

I. Introduction

Schizophrenia is a severe and disabling disorder affecting approximately 1% of the US population. Unfortunately, many patients do not achieve optimal symptom reduction with first line antipsychotic agents, and clozapine is the only antipsychotic approved by the FDA for people with treatment-refractory schizophrenia [1]. Sixty percent of those treated with clozapine, however, fail to adequately respond. Minocycline, a tetracycline antibiotic, crosses the blood-brain barrier, and possesses anti-inflammatory and neuroprotective

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Conflict of Interest: Deanna L. Kelly, PharmD, is a consultant for Lundbeck and XOMA. Joseph P. McEvoy, MD, is a consultant for Ameritox, Alkermes, Envivo, Jazz, Otsuka, and Merck. Robert P. McMahon, PhD, is a consultant for Amgen, Inc. Robert W. Buchanan, MD, is a Data Safety Monitoring Board member for Otsuka and Pfizer. He consulted with Abbott and is affiliated with Amgen, Bristol-Meyers Squibb, EnVivo, Omeros, and Pfizer. He is also part of the advisory boards of Abbott; Amgen; EnVivo; Janssen Pharmaceutical, Inc; NuPathe, Inc; Pfizer; Roche; and Takeda. The remaining authors declare no conflicts of interest.

Compliance With Ethical Standards: Research Involving Human Participants: This study was approved by the University of Maryland, Baltimore, the State of Maryland Department of Health and Mental Hygiene, and Duke University IRBs and performed in compliance with Declaration of Helsinki.

Informed Consent: Informed consent was obtained from all individual included participants.

properties that may have a therapeutic role in schizophrenia[2]. The potential psychotropic effects of minocycline were first noted in 2007 through case reports [3]. Since that time, some improvement in positive and negative symptoms with minocycline has been reported in a handful of clinical trials in Japan, Israel and elsewhere. In addition, our group and others have recently found minocycline to have beneficial effects on working memory, avolition, and anxiety/depressive symptoms in people receiving clozapine with refractory psychotic symptoms [4-7]. A recently published meta-analysis reported significant effects of adjunctive minocycline vs placebo for positive, negative, and general symptoms of schizophrenia, although no significant differences were found for neurocognitive function [8].

Minocycline is mainly metabolized via hydroxylation and N-demethylation by CYP450 enzymes, including CYP3A4, while clozapine is metabolized mainly by CYP1A2 through oxidative processes and secondarily by CYP3A4, CYP2D6, CYP2C9, and CYP2C19[9-11]. Clozapine is converted to norclozapine (generally considered the main metabolite) by N-demethylation[12], to clozapine-N-oxide at least partially by flavin-containing monooxygenase (FMO), and to glucuronides by the UDP glucuronosyltransferases (UGTs) [13]. Total plasma clozapine levels (clozapine and norclozapine) are frequently examined to assess potential for optimal response. Although little pharmacokinetic data exists, minocycline has been reported to increase exposure to theophylline, leading to theories that it may potentially inhibit CYP1A2[14, 15, 10, 11]. To date, no pharmacokinetic interaction has been reported between minocycline or other tetracycline agents and clozapine.

This paper examines clozapine plasma levels measured during our double-blind, placebo controlled 10-week study[4] of adjunctive minocycline to clozapine in treatment refractory schizophrenia.

II. Methods

This is a secondary analysis of a NIMH-funded study (R21MH091184-01A1) conducted at the Maryland Psychiatric Research Center and Duke University, therefore only pertinent methodological information will be included.

Participants

Participants were included if they (1) had a DSM-IV-TR diagnosis of schizophrenia or schizoaffective disorder, (2) were on a current dose of clozapine 200 mg/day, and (3) had a documented clozapine blood level of 350 ng/mL. Participants were excluded if they (1) were receiving treatment with tetracyclines or any derivatives or (2) had a previous known hypersensitivity to tetracyclines. Participants were required to have been on any adjunctive medications for at least two months and current doses for at least one month to qualify for inclusion. Doses of adjunctive medications and cigarette consumption were to remain fixed during the study, and no medications that impacted CYP1A2 enzyme activity were allowed introduction during the study.

Procedure

This study was approved by the University of Maryland, Baltimore, the State of Maryland Department of Health and Mental Hygiene, and Duke University IRBs and performed in compliance with Declaration of Helsinki. The study was registered in clinicaltrials.gov (NCT01433055) and was monitored by a Data Safety Monitoring Board. Participants were included if they successfully passed the Evaluation to Sign Consent (ESC).[16] Detailed information regarding inclusion criteria, study design, and informed consent has been published elsewhere. [4] After a 3-week screening and stabilization phase, participants were randomized to receive minocycline or matching placebo for 10 weeks. Dose of intervention was a 50 mg capsule twice daily for 1 week, and then increased to 100 mg capsule twice daily for the remainder of the study. Demographic information, clozapine plasma levels, and cigarette consumption were collected at baseline, and clozapine plasma levels were obtained every two weeks. Clozapine plasma levels were analyzed by LabCorp using liquid chromatography-tandem mass spectrometry (LC/MS-MS).

Data analysis

Changes from baseline were calculated for all available total clozapine, clozapine, and norclozapine plasma levels on study treatment and compared between the two treatment groups using a mixed model for analysis of variance (ANCOVA) for incomplete repeated measures, adjusting for baseline clozapine levels.

III. Results

Participants

Of the 73 participants screened, 52 were randomized to minocycline (n=29) or placebo (n=23). Two minocycline participants discontinued the 10-week study due to unrelated medical conditions. Twenty-eight participants randomized to minocycline and 22 to placebo were included in the current report. Demographic and medication information are presented in Tables 1 and 2. The two treatment groups were closely similar in demographics, clozapine dose, and all components of clozapine plasma levels at baseline. Randomization was not stratified by smoking status in the overall study, although it was at the MPRC site; by chance, more smokers were included in the minocycline group (n=15) compared to the placebo group (n=5). Among the smokers, no significant differences were found in number of cigarettes smoked at baseline or endpoint between the two groups. Per protocol requirements, cigarette smoking frequency remained consistent during the study, and smoking did not change significantly during the study. No participants stopped smoking during the trial. Clozapine was maintained at a constant dose throughout the study with the exception of one participant in the minocycline group whose clozapine total daily dose increased from 550 mg to 600 mg daily during the seventh week of the 10-week study. Concomitant medications were also maintained throughout study.

Clozapine plasma levels and associated changes

Clozapine, norclozapine, and total clozapine blood levels were obtained throughout the study for all participants. These are depicted in Figures 1a-c. Changes in plasma levels were

noted upon first measurement after initiation of randomized treatment (at 2 weeks). Week-to-week variations in the size of treatment differences (treatment by week interactions) were not statistically significant, suggesting a mechanism of rapid onset that was sustained throughout the 10-week study. Estimated average differences from baseline in clozapine plasma levels were significantly higher in the minocycline group when compared to the placebo group from repeated measures model. No statistically significant difference in plasma level was found between the two intervention groups for norclozapine; total clozapine level differences were not statistically significant, but not conclusively ruled out ($p=0.053$). Finally, we also examined the ratio of clozapine to norclozapine and found no significant differences between groups. ($F=3.41$, $df\ 1,46.8$, $p=0.07$); however this trend toward significance may suggest inhibition of clozapine metabolism by minocycline.

We also secondarily examined the relationship of clozapine plasma levels to clinical response, following improvements that were noted in an aspect of negative symptoms (e.g., avolition) with minocycline compared to placebo in a previous publication [4]. Avolition results were not explained by changes in clozapine blood levels; the changes in clozapine blood levels showed no significant correlation with changes in avolition. Interestingly, in the minocycline group increases in clozapine levels from baseline to week 10 were associated with higher total scores on the SANS negative symptom scale (Spearman's $R=0.51$, $p=0.006$). No significant correlation between clozapine levels and negative symptom changes was seen in the placebo group. This interaction between negative symptoms and clozapine levels was confirmed by Fisher's Z-test for difference in correlations ($z=2.59$, $p<0.01$). There is no evidence that improvement in any symptom domain is due to higher clozapine levels (total Brief Psychiatric Rating Scale $z=-.74$, $p=0.46$). Changes in norclozapine blood levels were not significantly correlated with changes in the SANS total score in either treatment group.

IV. Discussion

To date, no pharmacokinetic interaction has been reported between minocycline or other tetracyclic agents and clozapine. In this 10-week study, serial clozapine plasma levels were obtained at baseline and every two weeks, creating an excellent opportunity to explore potential pharmacokinetic interactions between clozapine and minocycline in a controlled setting. Our study demonstrated increases in clozapine plasma levels after the initiation of minocycline compared to the placebo group. The timeline of the increase, which appeared at the first post-intervention initiation blood draw, demonstrates a pattern consistent with that of Cytochrome P450 inhibition effects. The average increase in clozapine plasma level for the minocycline-treated group was 101.4 ng/ml, approximately a 21% increase from baseline. The corresponding changes in norclozapine and total clozapine levels between the groups were not statistically significant. It is hypothesized that CYP1A2 is the major enzyme responsible for the conversion of clozapine to norclozapine; however, CYP3A4 also contributes to production of this metabolite. This may at least partially explain the lack of difference in norclozapine metabolite plasma levels, if minocycline only affects one of these pathways.

Limitations of this study include that smoking and treatment interactions were not analyzed in great detail due to imbalance of distribution of smokers between the groups. As there were three times as many smokers in the minocycline group compared to the placebo groups, we would not have been able to test for a reliable interaction. The protocol stipulated, however, that smoking as well as concomitant medications were to remain constant without changes during the study to minimize possible smoking effects on changes in symptoms or clozapine plasma concentrations. Large decreases in tobacco smoking, which would be necessary to impact clozapine metabolism, were not observed during this study. Although one participant in the minocycline-treated group had a 50 mg clozapine dose increase during the study, this change did not occur until well after the increase in plasma clozapine levels were noted in the minocycline-treated group. Additionally, in several cases, blood was drawn only a couple of hours after the last clozapine dose, thus not reflecting true trough levels. In addition, only the clozapine and norclozapine levels were measured and reported. It is possible that changes in other metabolites such as clozapine n-oxide may have given additional information into this potential interaction. Lastly, while adherence to study drug was monitored for all participants by standard methods (pill counts), it is possible that participants may have developed differences in clozapine adherence throughout the study.

V. Conclusion

This analysis suggests that minocycline may contribute to increases in clozapine plasma levels. Further study is needed to examine possible explanations, such as minocycline's enzyme inhibitory properties, for the increase in clozapine levels among the minocycline group versus the placebo group.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research boards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Biography

Dr. Heidi Wehring and Dr. Teresa Elsobky share first authorship of this paper. Dr. Wehring is an Assistant Professor of Psychiatry, Maryland Psychiatric Research Center, University of Maryland School of Medicine. Dr. Teresa Elsobky is Assistant Professor, Shenandoah University School of Pharmacy.

1a

Total clozapine plasma levels (ng/ml)

* $p=0.053$, $F=3.96$, $df\ 4, 42.6$

1b

Clozapine plasma levels (ng/ml)

* $p=0.033$, $F=4.82$, $df=1, 46.2$

1c

Norclozapine plasma levels (ng/ml)

$p=0.754$, $F=0.10$, $df=1, 45$

Figure 1.

1a. Total clozapine plasma levels (ng/ml)

1b. Clozapine plasma levels (ng/ml)

1c. Norclozapine plasma levels (ng/ml)

Table 1
Participants' Demographic Information

	Minocycline (N=28)	Placebo (N=22)
Age (years; mean, sd)	42.9 (14.2)	42.5 (11.2)
Race, n (%)	White/Caucasian 18 (64%)	White/Caucasian 12 (55%)
	Black/African American 8 (29%)	Black/African American 8 (36%)
Number of male patients	20 (71%)	17 (77%)
Age of Illness Onset (years; mean, sd)	17.8 ± 6.5	18.6 ± 5.6
Inpatients, n (%)	11 (39%)	6 (27%)
Current smoker *, n (%)	15 (54%)	5 (23%)
Number of cigarettes smoked daily (mean, sd)	8.1 (5.5)	13.0 (5.7)

*
p<0.05

Table 2
Participants' Baseline Medication Information

	Minocycline (N=28)	Placebo (N=22)
Clozapine dose (mg/day; mean, sd)	432.1 (89.5)	430.7 (142.7)
Total Clozapine level (ng/mL; mean, sd)	777.4 (392.8)	845.6 (316.8)
Clozapine (ng/mL; mean, sd)	489.5 (257.1)	535.8.7 (197.1)
Norclozapine (ng/mL; mean, sd)	287.9 (165.0)	309.9 (168.3)
Other Antipsychotics, n (%)	13 (46%)	8 (35%)
FGA, n (%)	4 (14%)	1 (5%)
SGA, n (%)	9 (32%)	6 (27%)