ORIGINAL ARTICLE

Proof-of-Concept Trial of an α7 Nicotinic Agonist in Schizophrenia

Ann Olincy, MD; Josette G. Harris, PhD; Lynn L. Johnson, PharmD; Vicki Pender, BS; Susan Kongs, BS; Diana Allensworth, BS; Jamey Ellis, BS; Gary O. Zerbe, PhD; Sherry Leonard, PhD; Karen E. Stevens, PhD; James O. Stevens, DVM, PhD; Laura Martin, MD; Lawrence E. Adler, MD; Ferenc Soti, PhD; William R. Kem, PhD; Robert Freedman, MD

Context: The α7 nicotinic acetylcholine receptor gene, *CHRNA7*, is associated with genetic transmission of schizophrenia and related cognitive and neurophysiological sensory gating deficits. Cognitive dysfunction is responsible for significant psychosocial disability in schizophrenia. Nicotine, a low-potency agonist at the α7 receptor, has some positive effects on neurophysiological and neurocognitive deficits associated with schizophrenia, which suggests that more effective receptor activation might meaningfully enhance cognition in schizophrenia.

Objectives: To determine if 3-[(2,4-dimethoxy) benzylidene]anabaseine (DMXB-A), a natural alkaloid derivative and a partial α7 nicotinic cholinergic agonist, significantly improves neurocognition, and to assess, by effects on P50 auditory evoked potential inhibition, whether its neurobiological actions are consistent with activation of α7 nicotinic receptors.

Design: Randomized, double-blind crossover trial of 2 drug doses and 1 placebo.

Setting: General clinical research center.

Patients: Twelve persons with schizophrenia who did not smoke and were concurrently treated with antipsychotic drugs. One person was withdrawn because of a transient decrease in white blood cell count.

Intervention: Administration of DMXB-A.

Main Outcome Measures: Total scale score of the Repeatable Battery for the Assessment of Neuropsychological Status and P50 inhibitory gating.

Results: Significant neurocognitive improvement was found on the Repeatable Battery for the Assessment of Neuropsychological Status total scale score, particularly for the lower DMXB-A dose compared with placebo. Effects were greater than those of nicotine in a similar study. Significant improvement in P50 inhibition also occurred. Patients generally tolerated the drug well.

Conclusions: An α7 nicotinic agonist appears to have positive effects on neurocognition in persons with schizophrenia. Longer trials are needed to determine the clinical utility of this novel treatment strategy.

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THE α7 NICOTINIC RECEPTOR was identified as a possible pathological mechanism and hence a potential therapeutic target in schizophrenia by convergent evidence from neurobiological and genetic studies. The neurobiological studies originated from the clinical observation that an elementary deficit in psychosis is inability to filter or gate response to sensory stimuli.[1] Physiologically, healthy control subjects have diminished amplitude of the P50 component of the evoked response to the second stimulus compared with the first of paired auditory stimuli because of inhibitory mechanisms activated during the response to the first stimulus. Persons with schizophrenia generally show less inhibition, a deficit correlated with impairment in sustained attention.[2,3] In animal models of the evoked potential response, cholinergic stimulation of α7 nicotinic acetylcholine receptors, which are found on presynaptic and postsynaptic sites on inhibitory interneurons of the hippocampus, is essential for this inhibition.[4-6] Failure of activation of α7 receptors is thus a putative neurobiological mechanism of attentional dysfunction in schizophrenia. The diminished expression of α7 nicotinic receptors in several regions of human postmortem brain tissue further supports the hypothesis of a deficiency in this aspect of cholinergic neurotransmission in schizophrenia.[7,8] Genome-wide linkage analysis, a strategy that is independent of the neurobiological hypothesis of a deficit in nicotinic cholinergic receptors, showed that the P50 gating deficit is heritable in families with schizophrenia. Maximal linkage is at the chromosome 15q14

Author Affiliations are listed at the end of this article.

locus of *CHRNA7*.[2] Polymorphisms in the gene and its nearby partial duplication are associated with diminished P50 inhibition and cognitive dysfunction.[9-11] Several subsequent studies have found evidence for linkage of schizophrenia to this site.[12-18] Thus, both lines of investigation point to a role of the α7 nicotinic receptor in the pathophysiology of sensory gating dysfunction in schizophrenia.

Nicotine is heavily abused by persons with schizophrenia, an observation that has sometimes been explained as an attempt at self-medication. About 80% of patients smoke, with a mean consumption of 30 cigarettes per day; per cigarette, they extract 50% more nicotine than other smokers.[19] Higher nicotine levels are consistent with activity at α7 receptors, which are less sensitive to nicotine than α4β2 nicotinic receptors, the other common neuronal nicotinic receptor that is found on presynaptic terminals of many different neuronal types. High doses of nicotine significantly improve P50 inhibition in patients and their relatives.[20] In addition, nicotine has positive neurocognitive effects in schizophrenia, particularly on attention, which are consistent with the relationship between diminished sensory gating and attention dysfunction. However, these effects, which are also observed in healthy subjects, are severely limited by tachyphylaxis and are not clinically significant.[21-26] Thus, although nicotine itself has no useful therapeutic effect, its actions have prompted investigation of less toxic and more effective agonists.

The drug 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) (**Figure 1**) is a derivative of the naturally occurring alkaloid anabaseine.[27] It is a partial agonist at human α7 nicotinic receptors, and at higher concentrations it is a weak antagonist at α4β2 receptors and serotonin 5-HT_3 receptors.[27-30] Approximately 40% of an oral dose is absorbed within 1 hour of administration.[31] Metabolites with hydroxyl substituents at positions 2 and 4 are more efficacious agonists when bound to the receptor, but to what extent they are produced in human brain is unknown.[30,31] Administration of DMXB-A improves memory in several animal models, and it normalizes inhibition of auditory responses in rodents, with significantly less tachyphylaxis than nicotine.[32,33] Positive neurocognitive effects, particularly on attention, were observed in healthy volunteers.[34]

The aims of this study were to determine, as a proof of concept, if DMXB-A significantly improves neurocognition in schizophrenia and to assess, by effects on P50 inhibition, whether its actions are consistent with activation of α7 nicotinic receptors. Because the proposed effect is agonism at a ligand-gated ion channel, biological effects were expected immediately, consistent with the results from animal models.[33] Therefore, single-day administration was chosen for an initial examination of effectiveness.

METHODS

TRIAL DESIGN

The first dose of DMXB-A was administered orally (150 or 75 mg), followed 2 hours later by a supplemental half dose (75 or 37.5 mg). The half dose, administered at the predicted half-life of the first dose,[34] extended the period of therapeutic drug levels during the behavioral measurements. Experimenters and subjects were both blinded to treatment identity until the completion of the study. Randomization was performed using a random number table by a pharmacist unaffiliated with the study. The study occurred from April 1 through August 30, 2004.

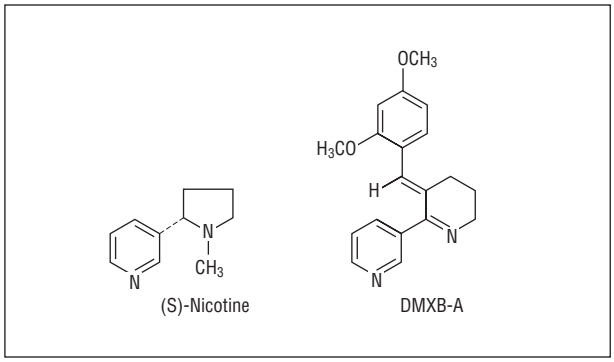

Figure 1. Comparison of the structure of nicotine and 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A).

The Repeatable Battery for Assessment of Neuropsychological Status (RBANS) was administered immediately after the second dose on each experimental day.[35] The primary neurophysiological measure was P50 auditory evoked potential suppression in response to repeated stimuli.[20] This measure was chosen because it had previously demonstrated effects of nicotinic agonism in schizophrenia and effects of DMXB-A in animal models.[20,33] Recordings were made each day before drug administration and twice after the first and once after the second dose. The Brief Psychiatric Rating Scale (BPRS) was administered after each electrophysiological recording. Plasma drug levels were measured 1.5 to 2.0 hours after each dose. An electrocardiogram, measurement of hematological parameters and clinical chemistries, and a checklist of adverse effects were completed at the end of each day.

The sample size of 12 patients was chosen based on power calculations from previous studies of the neurophysiological effects (effect size, 0.8) of nicotine in schizophrenia.[20] The study was approved by the University of the Colorado multi-institutional review board and the US Food and Drug Administration.

SELECTION AND DIAGNOSIS OF SUBJECTS

Eighteen patients underwent screening for participation. Thirteen were medically eligible and consented to the study. One subject had a grand mal seizure while signing the consent form; she did not participate further in the study and was not included in the analysis. Subjects were interviewed with the Structured Clinical Interview for *DSM-IV* Axis I Disorders or the Diagnostic Interview for Genetic Studies and met *DSM-IV-TR* criteria for schizophrenia. Subjects were selected to be nonsmokers for at least 1 month to avoid the possibility of interaction with long-term exposure to nicotine and were concurrently treated with neuroleptic medications (**Table 1**).

PREPARATION OF DMXB-A

The DMXB-A was synthesized as a dihydrochloride salt by reaction of synthetic anabaseine dihydrochloride with 2,4-dimethoxybenzaldehyde according to a recently published procedure.[27] (All reagents were obtained from Aldrich Chemical Co, Milwaukee, Wis.) The product was obtained as a yellow solid (melting point, 215°C -219°C [decompose]). The C18 high-performance liquid chromatography elution profile of the syn-

Table 1. Demographic and Treatment Information

Sex/ Age, y	Education, y	Neuroleptic Treatment
F/42	16	Risperidone, 6 mg
M/47	11	Fluphenazine decanoate, 25 mg, for 2 wk
M/45	16	Olanzapine, 20 mg
M/35	13	Aripiprazole, 30 mg
F/46	16	Quetiapine, 800 mg
M/20	14	Risperidone, 8 mg; olanzapine, 10 mg
F/58	20	Aripiprazole, 15 mg; thioridazine, 50 mg
M/50	14	Olanzapine, 20 mg; thioridazine, 100 mg
M/47	16	Olanzapine, 15 mg; aripiprazole, 10 mg
M/50	16	Ziprasidone, 200 mg
F/48	16	Quetiapine, 600 mg
M/46	15	Risperidone, 4 mg

thetic product indicated a purity exceeding 99% with no specific contaminants. The proton nuclear magnetic resonance, mass, and infrared spectra of the DMXB-A sample were consistent with previously obtained data for DMXB-A samples used in earlier studies.[31,34] The elemental analysis was as follows: Calculated for $C_{19}H_{20}N_2O_2 \cdot 2HCl$, C, 59.85; H, 5.82; N, 7.35; found, C, 59.63; H, 5.78; N, 7.23. The synthetic sample was stored in the dark at room temperature in a tightly sealed amber glass bottle before it was encapsulated. Identical-appearing placebo and drug capsules were prepared.

PLASMA DRUG LEVELS

Levels of DMXB-A in subjects' plasma were quantitatively determined by a modification of previously reported high-performance liquid chromatography methods.[31] Internal standard (50 ng of GTS-83, a DMXB-A analogue with very similar chemical properties dissolved in 0.10 mL of buffer A) was added to the plasma sample (0.50 mL), and then 0.50 mL of buffer A (50mM ammonium acetate [pH, 4.50]) was added. After mixing, the resulting suspension was placed on a cartridge (Bond-Elut C18; Varian Inc, Palo Alto, Calif; 200 mg; 3-mL size) that had been preequilibrated with methanol, water, and finally buffer A. After washing twice with 2.5 mL of buffer A and 2.5 mL of 20% methanol in water, DMXB-A was eluted with three 2.0-mL washes of 0.50% ammonium hydroxide in methanol. The 3 washes were combined and dried in a vacuum (SpeedVac; Telechem International, Inc, Sunnyvale, Calif) in the absence of light. The resulting sample was dissolved in 0.45 mL of buffer A, filtered by centrifugation, and injected into a C18 column (Ultrasphere ODS; Beckman Coulter, Fullerton, Calif; 4.6-mm diameter; 250-mm length). After an initial minute of elution with the column preequilibrated with a solution consisting of 20% acetonitrile and 50mM ammonium acetate (pH, 4.5), the sample was resolved using a 20-minute 20% to 60% linear gradient of acetonitrile, also in 50mM ammonium acetate (pH, 4.5). The internal standard eluted at about 10.8 minutes; DMXB-A, at about 12.9 minutes. The hydroxy metabolites eluted earlier (the 2,4-dihydroxy metabolite at approximately 3.8 minutes, the 4-hydroxy metabolite at 6.3 minutes, and the 2-hydroxy metabolite at 8.7 minutes) and before the internal standard as previously reported.[27] The DMXB-A and synthetic samples of the hydroxy phase 1 metabolites were completely resolved by the high-performance liquid chromatography method and were measured by their 400-nm absorbance areas relative to that of the internal standard. Recoveries of the compounds including internal standards from plasma samples generally exceeded 80%. The identity of each compound peak was based not only on its characteristic retention time but also on the wavelength of its long wavelength peak absorbance recorded using a diode array detector. Plasma samples were extracted and analyzed 3 to 5 times.

NEUROPSYCHOLOGICAL ASSESSMENT

The RBANS has a total scale score and 5 index scores. The scores are scaled with a mean of 100 and an SD of 15. Two alternate test versions were used; each subject received 2 test sessions with 1 version and 1 session with the alternate version, in random order. No subject received the same version on successive days. Persons with schizophrenia score significantly lower than controls on this measure; the decrement correlates with diminished psychosocial status.[35] The intraclass correlation for repeated testing in schizophrenia is 0.83 for the total scale score.[35] Phase 1 trials generally do not have predetermined criteria for successful treatment. However, a 15-point change in total scale score would be equivalent to the mean difference between persons with schizophrenia who are employed and unemployed in the reference sample assessed to validate the RBANS for use in schizophrenia.[35] A subsequent analysis of another reference sample showed that a 10-point or greater increase on repeated testing without any change in treatment would be expected in only 8% of patients or 1 subject in the current study[36]

ELECTROPHYSIOLOGICAL RECORDINGS

Auditory stimuli were presented in a conditioning testing paradigm with an intrapair interval of 0.5 second and interstimulus interval of 10 seconds. Electroencephalographic (EEG) activity was recorded from a gold disk electrode affixed to the vertex. Data from the EEG and electro-oculogram were collected for 1000 milliseconds for each paired stimulus presented by a technician (J.E.) blinded to the subjects' treatment. Trials were rejected during recording if they contained muscle startle artifact or eye blinks as indicated by an EEG or electro-oculographic voltage of ±30 μV at 50 milliseconds after stimulus or if there was evidence of drowsiness in the EEG, as indicated by repetitive waves greater than 20 μV. Inhibition of P50 is impaired during non–rapid eye movement sleep because of altered cholinergic neuron activity.[37] The mean number of accepted responses did not differ between treatment conditions (49%-54%).

A computer algorithm was used to identify and quantify the P50 wave.[2] The P50 test–conditioning ratios were calculated by dividing the test P50 amplitude by the conditioning P50 amplitude. The variability between the 3 recordings performed after drug administration during the same day was considerable; the small number of response pairs, usually 16, likely contributed to the variability, as the signal-to-noise ratio increases as the square root of the number of trials. Therefore, grand averages of the 3 recordings obtained after each treatment were used for the final analysis. The distribution of the variables was tested for normality by a goodness-of-fit χ^2 test; there were no significant deviations (conditioning amplitude, P=.40; test amplitude, P=.95; ratio, P=.13).

STATISTICAL ANALYSIS

The RBANS data were analyzed by a repeated-measures, mixed-effects analysis of variance (SAS ProcMix; SAS Institute Inc, Cary, NC) for a carryover study, with treatment regimen, visit day, and sequence of treatments as fixed effects. The model was validated by permitting an arbitrary multivariate 3×3 covariance matrix of random period (visit) effects. Estimates of the correlation coefficients between visits ranged from 0.810 to 0.847, and estimates of the SDs varied from 9.59 to 11.41, consistent

Table 2. DMXB-A and Metabolite Plasma Concentrations*

Plasma Concentration, ng/mL	Low-Dose DMXB-A		High-Dose DMXB-A	
	75 mg	37.5 mg	150 mg	75 mg
DMXB-A	13.2 ± 14.4	10.9 ± 13.4	23.2 ± 16.0	19.7 ± 17.5
4-OH DMXB-A	8.8 ± 6.6	6.5 ± 3.5	21.4 ± 8.1	19.5 ± 11.0

Abbreviations: DMXB-A, 3-[(2,4-dimethoxy)benzylidene]anabaseine; 4-OH, DMXB-A metabolite.
*Data are expressed as mean ± SD.

with sphericity. Tests comparing differences in log likelihood found no improvement for the arbitrary multivariate model, compared with a model assuming compound symmetry. The 2 planned comparisons for the effects of either dosing level of DMXB-A on total scale score compared with placebo were performed by *t* tests appropriate for a priori contrasts within the analysis of variance, using its least squares estimates of mean and standard error. The P50 data were analyzed by a multivariate analysis of variance. Effects on the BPRS were analyzed by Wilcoxon signed rank tests. Relationships between neurocognitive and electrophysiological effects were tested using a multiple regression with placebo values as a baseline variable. For all analyses, comparisons of results on subscales were subjected to Bonferroni correction. For all tests, the criterion for significance was $\alpha = .05$ (2-tailed).

RESULTS

DRUG ABSORPTION AND METABOLISM

The plasma levels were consistent with the pharmacokinetic parameters established in the previous phase 1 study in healthy controls[34] (**Table 2**). The plasma levels after the first dose were not significantly different from those after the supplemental half dose on each day, which suggests that a relatively stable concentration of drug was reached. The level of the principal metabolite of DMXB-A, 4-OH DMXB-A, was measured as well (**Figure 2**). The apparent saturation of the 4-OH DMXB-A metabolite levels in patients with higher DMXB-A levels suggests that biotransformation, which accounts for more than 99% of DMXB-A elimination, is slower in some individuals, which results in higher levels of DMXB-A and lower levels of 4-OH DMXB-A.[31,34]

NEUROCOGNITIVE EFFECTS

The neurocognitive effect of drug treatment as measured by the RBANS total scale score was significant for the effect of treatment ($F_{2,21} = 3.47$; $P = .050$). Effects of visit number, indicative of practice effect on the RBANS, and treatment order, indicative of possible carryover effects of the treatment during the previous visit, were also examined. Visit number, but not treatment order, had significant effects. Entering visit effect into the analysis lowered the significance level for treatment effect ($F_{2,19} = 2.95$; $P = .08$). Specific contrasts were tested for each of the 2 dosing levels of DMXB-A, compared with placebo. Only the lower DMXB-A dosing level significantly improved overall performance (total scale score $t_{19} = 2.42$; $P = .03$;

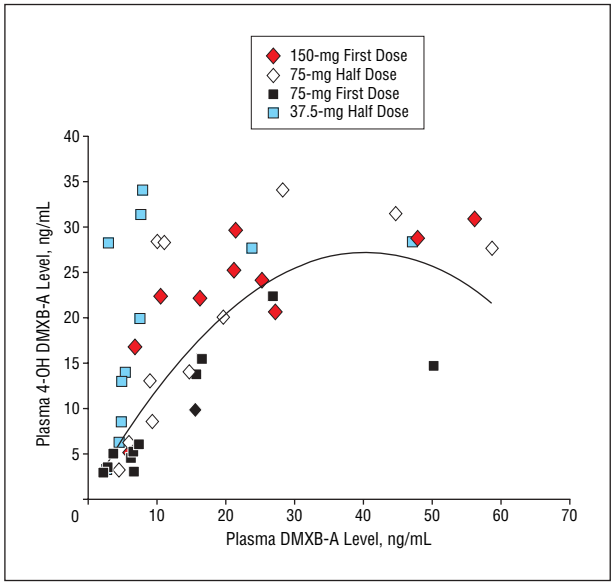

Figure 2. Relationship between plasma levels of 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) and its metabolite, 4-OH DMXB-A. A quadratic polynomial model for all values is shown ($r_{42} = 0.77$; $P < .001$).

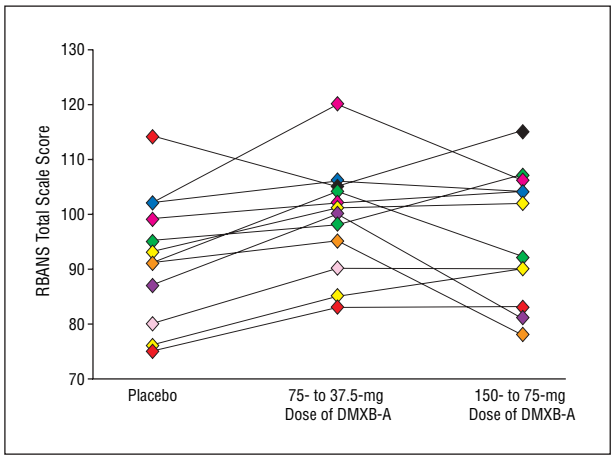

Figure 3. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) total scale scores during placebo and 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) treatment.

Figure 3 and **Table 3**). However, the analysis did not establish a significant difference between the 2 doses. Among the indices, the attention index showed the largest improvement; none of the effects on the individual indices were significant after Bonferroni correction for multiple testing (**Figure 4**).

Previous comparison between employed and unemployed patients in a reference sample showed a mean 15-point difference in RBANS total scale score for employed patients.[35] In this study, 5 subjects increased their total scale score from 12 to 18 points during DMXB-A administration compared with their placebo values, whereas only 1 change of this magnitude is expected to occur during repetition of the test alone without change in treatment[36] (goodness of fit $\chi^2_1 = 14.28$ with Yates correction; $P < .001$).

Table 3. Neurocognitive Effects of DMXB-A in Schizophrenia*

RBANS Indices	Placebo	DMBX-A Dose 75 and 37.5 mg	DMBX-A Dose 150 and 75 mg
Total scale score†	93.5 ± 3.05	98.8 ± 3.10	95.6 ± 3.01
Attention	89.6 ± 3.89	97.8 ± 4.00	92.2 ± 3.79
Immediate memory	99.9 ± 4.42	103.4 ± 4.53	102.1 ± 4.32
Delayed memory	97.5 ± 3.91	101.2 ± 4.06	96.0 ± 3.77
Visuospatial/construction	97.8 ± 5.12	101.8 ± 5.22	101.9 ± 5.03
Language	91.9 ± 2.65	93.7 ± 2.15	92.3 ± 2.56

Abbreviations: DMXB-A, 3-[(2,4-dimethoxy)benzylidene]anabaseine; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

*Data are expressed as mean ± SE by least squares estimate.

†Difference of DMXB-A, 75 and 37.5 mg, from placebo, $t_{19} = 2.42$ (P=.03); effect size is 0.51.

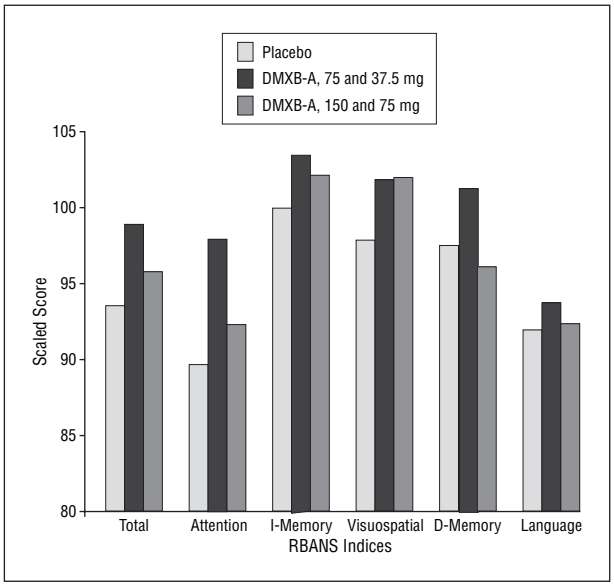

Figure 4. Effects of 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) and placebo on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) total scale score and its specific indices. D indicates delayed memory; I, immediate.

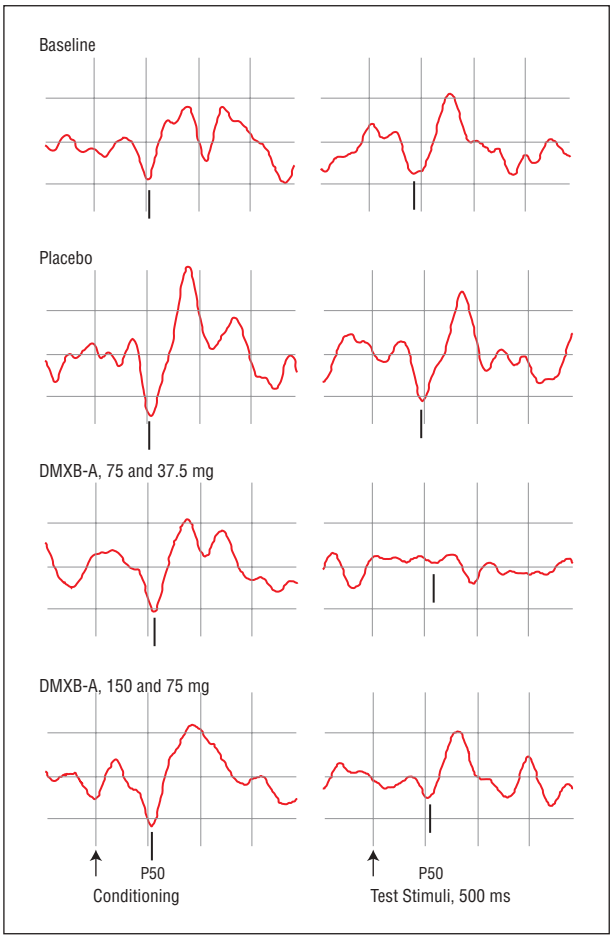

Figure 5. Auditory evoked responses of a subject with schizophrenia. Stimuli were a conditioning auditory stimulus and an identical test stimulus, delivered 500 milliseconds apart. Inhibition of the test P50 response is increased by the administration of 3-[(2,4-dimethoxy)benzylidene] anabaseine (DMXB-A), particularly during the lower dose (third row) compared with the baseline (before drug administration) and placebo responses above it. Arrows show the timing of the stimuli, and vertical bars mark the location of the P50 wave in the tracings above. Positive polarity is downwards; vertical grid interval, 2 μV; and horizontal grid interval, 50 milliseconds.

NEUROPHYSIOLOGICAL EFFECTS

Unlike neurocognition, which was measured only during drug exposure, P50 suppression was measured before drug administration on each day; these baseline values were not significantly different, consistent with the lack of repetition effects on this measure. During drug treatment, the amplitude of the P50 response to the first or conditioning stimulus was not significantly different during the 3 treatment conditions. However, the amplitude of the second or test response was significantly decreased during the low dose compared with placebo ($F_{1,11}$=6.46; P=.03; **Figure 5**). There was no significant difference in effect between the 2 doses. The decrease in amplitude of the test response at the low dose was also reflected as a significant difference in the P50 test amplitude–conditioning amplitude ratio ($F_{1,11}$=9.69; P=.01; **Table 4**). Thus, DMXB-A significantly increases inhibition of the test response. The mean ± SD P50 ratio measured after the lower DMXB-A dose was 0.30 ± 0.22 compared with 0.73 ± 0.51 after placebo. The P50 ratios during placebo were within the range previously observed in schizophrenia, whereas the P50 ratios during lower-dose administration were within the range observed in controls.[2] The decrease in P50 ratio between low-dose treatment and placebo correlated nonsignificantly with the increase in the RBANS total scale score (r_{10}=–0.56; P=.06).

Relationships between the plasma level of DMXB-A and RBANS total scale score and indices or P50 inhibition were nonsignificant (**Figure 6**). One of the 2 subjects whose RBANS total scale score decreased at the higher dose compared with placebo had the highest plasma DMXB-A levels; the other had levels comparable to the mean levels at this dose.

CLINICAL EFFECTS

Symptomatic measures were not expected to change during a brief trial; therefore, clinical ratings on the BPRS

Table 4. Neurophysiological Effects of DMXB-A in Schizophrenia*

	P50 Conditioning Amplitude, μV	P50 Test Amplitude, μV	P50 Ratio†
Placebo			
Baseline	3.52 ± 1.84	2.74 ± 1.50	0.83 ± 0.27
Treatment	2.94 ± 1.32	1.68 ± 0.75	0.73 ± 0.51
DMXB-A, 75 and 37.5 mg‡			
Baseline	3.14 ± 2.19	2.74 ± 1.64	0.92 ± 0.32
Treatment	3.50 ± 1.80	0.92 ± 0.87	0.30 ± 0.22§
DMXB-A, 150 and 75 mg			
Baseline	3.13 ± 1.68	3.09 ± 1.39	1.05 ± 0.43
Treatment	3.32 ± 1.46	1.29 ± 1.30	0.60 ± 0.84

Abbreviation: DMXB-A, 3-[(2,4-dimethoxy)benzylidene]anabaseine.
*Data are expressed as mean ± SD.
†Calculated as test amplitude/conditioning amplitude.
‡Calculated as multivariate repeated-measure analysis of variance, contrasts comparing DMXB-A, 75 and 37.5 mg, with placebo (adjusted for baseline). For univariate analyses, P50 test amplitude, $F_{1,11} = 6.46$ (P=.03), and P50 ratio, $F_{1,11} = 9.69$ (P=.01); for bivariate analysis, P50 conditioning and test amplitudes, $F_{2,10} = 4.92$ (P=.03).
§For comparison with baseline, t test for paired samples, $t_{11} = 5.50$ ($P<.001$).

were considered exploratory pilot data. Ratings decreased in the course of the experimental day for all 3 treatments (**Figure 7**). The only significant change from placebo ratings was during the higher-dose treatment, after the second half dose (Wilcoxon signed rank test, P=.047). Changes after DMXB-A administration were in ratings of blunted affect, somatic concerns, guilt, grandiosity, hallucinations, anxiety, and disorganization. The most commonly reported adverse complaint was somnolence, which occurred in 7 patients receiving the higher drug dose, compared with 6 receiving placebo and 3 receiving the lower dose (**Table 5**). This adverse effect may be consistent with performance of the study in a general hospital room. The patients were not noted to be sedated, and their EEG recordings after drug treatment showed only the amount of drowsiness generally experienced during this procedure. There was no relationship between this complaint and the P50 ratio (r=0.002).

Additional comments of the patients during the BPRS evaluations were noted. All comments came during high-dose treatment. Two patients spontaneously remarked that the drug helped them think more clearly and maintain concentration. Both of them, one a computer programmer and the other a literary translator, felt energized to do these tasks, which they had not been able to accomplish recently. On questioning, one of them distinguished this positive effect from that of clozapine, which had been helpful to her, and that of cigarettes, which she had smoked earlier in her life. A third patient, during her final BPRS rating, expressed surprise that her voices had shrunk to whispers, which was rare for her. She also felt that her concentration level had increased.

SAFETY EVALUATION

One patient had to be withdrawn from the study when his white blood cell count, which had been 3900/μL after placebo, fell to 2900/μL after the higher drug dose. The patient did not become ill, and his white blood cell count had returned to normal when he was seen again 3 days later.

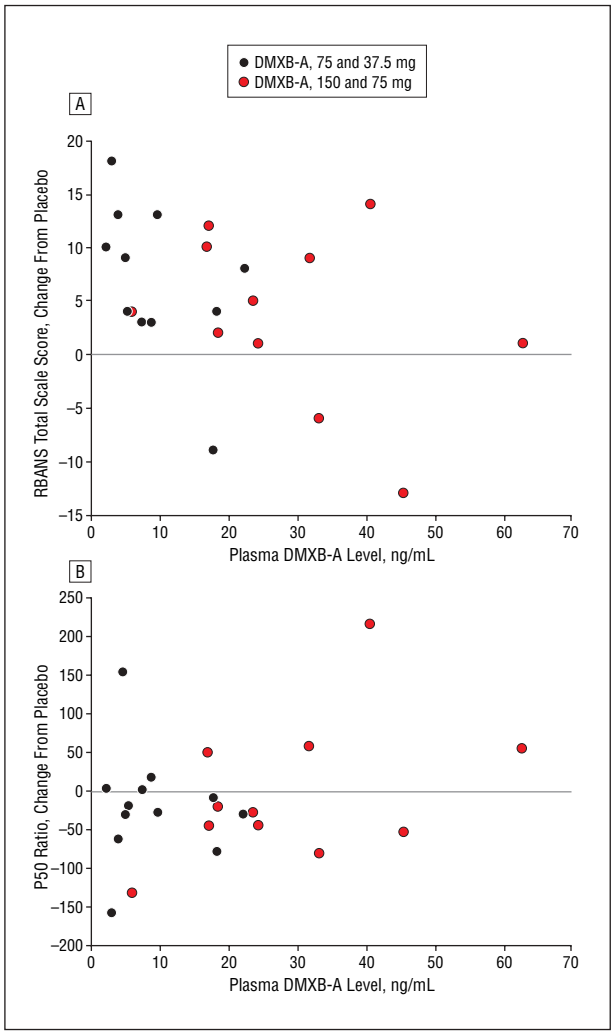

Figure 6. Plasma levels of 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) and changes in Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) total scale score (A) and P50 ratio (B) from placebo. Neither of these parameters was significantly correlated with plasma level at either dose.

COMMENT

The α7 nicotinic agonist DMXB-A had a positive effect on neurocognition in schizophrenia. The effects for some patients were sufficiently robust (8- to 12-point increase in RBANS total scale score) to be suggestive of the possibility of a meaningful clinical effect. There was an accompanying increase in the suppression of the P50 auditory evoked potential to repeated stimuli. However, the clinical relevance of the changes observed herein requires assessment after longer-term administration. The purpose of this study was to determine if there is sufficient evidence of effect to support such a study.

A comparator is the effects of nicotine, which a separate study had earlier examined in a similar group of 10 patients who were nonsmokers.[21] Like DMXB-A, nicotine increased performance on the RBANS attention in-

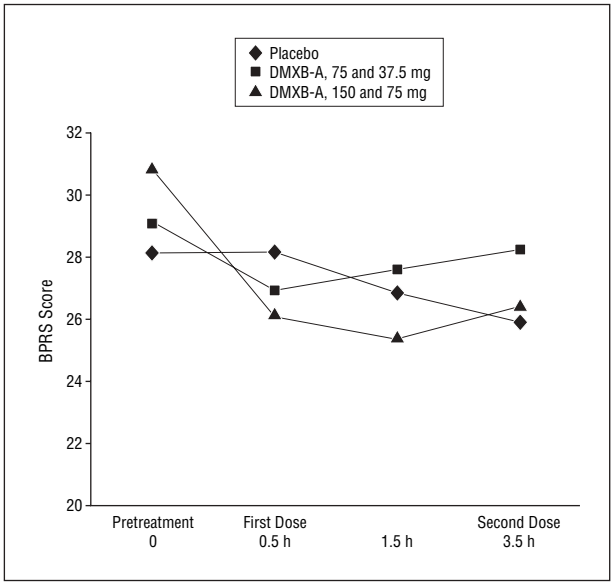

Figure 7. Effects of 3-[(2,4-dimethoxy)benzylidene]anabaseine (DMXB-A) and placebo on Brief Psychiatric Rating Scale (BPRS) scores. At 1.5 hours, the ratings for the higher dose are significantly different from those for placebo (Wilcoxon matched pairs, *P*=.047).

Table 5. Patients' Self-ratings of Adverse Effects

Body System by Adverse Effect	No. of Patients		
	DMXB-A, 75 mg (n = 11)	DMXB-A, 150 mg (n = 12)	Placebo (n = 12)
Central and peripheral nervous systems			
Headache	2	0	1
Dizziness	0	0	1
Tremor	1	2	2
Psychiatric			
Nervousness	3	1	4
Somnolence	3	7	6
Paranoia	2	1	1
Body as a whole			
Fatigue	2	2	2
Back pain	0	1	0
Asthenia	1	1	1
Renal			
Urinary urgency	1	0	1
Gastrointestinal tract			
Diarrhea	0	0	1
Flatulence	0	0	4
Cardiovascular			
Chest pain	0	0	1
Skin and appendages			
Rash	2	1	1
Itching	0	0	1
Sweating	0	1	0

dex; however, effects were smaller and less significant with nicotine. In addition, nicotine diminished performance on the immediate and delayed memory indices, whereas DMXB-A nonsignificantly increased performance on both of these indices. Thus, the total scale score was significantly increased with DMXB-A. In contrast, for nicotine the total scale score did not change (mean ± SD, 88.4 ± 13.0 compared with 89.2 ± 16.3 with placebo).[21]

Increased inhibition of the P50 response during DMXB-A administration supports the hypothesis of an agonist effect on α7 receptors located on inhibitory interneurons. In animal models, stimulation of α7 nicotinic receptors with DMXB-A selectively decreases the test-response amplitude, as was found in this study.[33] The data thus suggest that inhibitory circuits, which are normally responsible for suppression of the test's response and appear to be dysfunctional in schizophrenia, may be partly intact and susceptible to activation by nicotinic agonists, as well as other treatments.

The finding of significant differences from placebo only at lower dose on the neurocognitive and neurophysiological parameters suggests that there may be tachyphylaxis, but a larger number of subjects is required to establish this point. At this stage of investigation, the difference between the 2 doses is not significant. Tachyphylaxis is a well-characterized property of most ligand-gated ion channels, including α7 receptors, and has been observed in clinical trials of nicotine and other nicotinic agonists.[21,38] In animal studies with DMXB-A, there was no tachyphylaxis to repeated dosing, but a nonsignificant indication of decreased effect at higher doses.[33] In the present study, plasma levels were maintained for approximately 3 hours by repeated dosing. The positive neurocognitive effects were observed during the latter half of this period, which suggests that sustained effects with α7 agonists are achievable. For DMXB-A, more prolonged effects might therefore be obtained by a slow-release formulation. Patients who smoked were excluded in this trial to prevent interference with the effects of DMXB-A by the effects of long-term exposure to nicotine. Potential interactions between the effects of long-term nicotine exposure and DMXB-A remain to be examined, including the possibility that DMXB-A could over time alter the patients' smoking behavior.

Therefore, a phase 2 trial of DMXB-A has been initiated, a double-blind 3-arm crossover study with 1-month administration of 2 doses (150 and 75 mg 2 times a day) and placebo. Smokers and nonsmokers are being studied. The National Institute of Mental Health Measurement and Treatment Research to Improve Cognition in Schizophrenia Neuropsychology Battery has been substituted for the RBANS. This study will help determine if the effects of DMXB-A are primarily on attention or extend to other cognitive domains as well. Study of the effects on neurocognition of the atypical neuroleptics shows a generally similar pattern of effects on total scale and attention scores.[39] Several compounds in current clinical use may also have direct or indirect effects on α7 nicotinic receptors. The anticholinesterase galantamine, which has additional modulatory effects on the α7 nicotinic receptor, has been reported to be beneficial for schizophrenia in case studies.[40] Tropisetron, a serotonin 5-HT_3 antagonist approved for use outside the United States as an antinausea drug, has nearly equal potency as an α7 nicotinic receptor agonist. Tropisetron also improves the inhibition of P50 responses in schizophrenia, perhaps through α7 nicotinic receptor agonism.[41] The atypical neuroleptic clozapine is not a direct nicotinic agonist, but it

indirectly increases release of acetylcholine in the hippocampus, a property not shared by older dopamine D_2 receptor antagonists.[42] Clozapine increases inhibition of the P50 auditory response in schizophrenia; animal models demonstrate mediation of this effect by stimulation of $\alpha 7$ nicotinic receptors.[43,44] Clinical response to clozapine is greater in patients who smoke before the initiation of treatment compared with those who do not, and it also results in decreased smoking behavior during treatment, perhaps consistent with nicotinic cholinergic agonism as a mechanism of the antipsychotic effect of clozapine.[45,46] The findings with DMXB-A in this study are a direct demonstration of the effects of activation of $\alpha 7$ nicotinic receptors in schizophrenia, and they suggest that nicotinic cholinergic agonism may be a therapeutic mechanism worthy of further development for schizophrenia.

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Author Affiliations: Department of Veterans Affairs Schizophrenia Research Center and Veterans Integrated Service Network 19 Mental Illness Research, Education and Clinical Centers (Drs Olincy, Harris, Johnson, Zerbe, Leonard, K. E. Stevens, J. O. Stevens, Martin, Adler, and Freedman; Mss Pender, Kongs, and Allensworth; and Mr Ellis), Departments of Psychiatry (Drs Olincy, Harris, Johnson, Zerbe, Leonard, K. E. Stevens, J. O. Stevens, Martin, Adler, and Freedman; Mss Pender, Kongs, and Allensworth; and Mr Elis), Preventive Medicine and Biometics (Dr Zerbe), and Pharmacology (Drs Leonard and Freedman), University of Colorado School of Medicine, Denver; and Department of Pharmacology and Toxicology, University of Florida School of Medicine, Gainesville (Drs Soti and Kem).
Correspondence: Robert Freedman, MD, Department of Psychiatry C268-71, University of Colorado Health Sciences Center, Denver CO 80262 (Robert.Freedman @UCHSC.edu).
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