Effects of Treatment With Etizolam 0.5 mg BID on Cognitive Performance: A 3-Week, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Two-Treatment, Three-Period, Noninferiority Crossover Study in Patients With Anxiety Disorder

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ABSTRACT

Background: Etizolam is an anxiolytic drug with a pharmacologic profile similar to that of the classic benzodiazepines. Neurochemical research suggests that etizolam may have selectivity for the subpopulation of γ-aminobutyric acid type A receptors associated with anxiety (ie, α1, β2, γ2). This property, plus its characterization as a ligand with fewer of the adverse events typical of full agonists (impaired cognitive function, tolerance, and dependence), led to its selection for this study.

Objectives: The primary aim of this study was to test for the noninferiority of etizolam 0.5 mg BID versus placebo in affecting cognitive function in patients with mild to moderate anxiety disorder of recent onset (<1 month). Anxiety measures and tolerability were also assessed.

Methods: Patients between the ages of 18 and 65 years were eligible for enrollment. This double-blind, placebo-controlled study was performed in 5 centers in Italy using a 2-treatment, 3-period crossover design. Patients were randomized to 3-week sequences of either etizolam-placebo-placebo or placebo-etizolam-etizolam. They were evaluated at 4 scheduled visits (screening and days 7, 14, and 21). Cognitive function was assessed using scores from the Wechsler Adult Intelligence Scale (WAIS) Digit Span test (total forward and backward scores and the time required to perform the test). Anxiety was measured using the Hamilton Anxiety Rating Scale (HAM-A) and the State-Trait Anxiety Inventory (STAI) for screening and to monitor adequacy of therapy. Blood pressure, heart rate, weight, and adverse events were also recorded.

Results: A total of 77 white patients were enrolled (mean age, 33.3 years [range, 22–60 years]; 62.3% female; mean weight, 65.2 kg). With a power of 0.80, the difference between the effects of etizolam and placebo on WAIS Digit Span performance was not significant for total score (0.102 [90% CI, –0.130 to 0.335]) or time required for completion (0.029 second [90% CI, –0.574 to 0.632]). Anxiety, as measured using the HAM-A and STAI instruments, did not differ significantly between groups. No significant differences were found between etizolam 0.5 mg BID and placebo for cardiovascular events, weight changes, or

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adverse events. Mild or moderate somnolence was reported by 7 of 77 patients (9.1% [3 patients while receiving etizolam and 4 patients while receiving etizolam and placebo]).

Conclusions: No significant differences between etizolam 0.5 mg BID and placebo were found for cognitive function or anxiety measures in these patients with anxiety. Etizolam was well tolerated. (*Clin Ther.* 2009;31:2851–2859) © 2009 Excerpta Medica Inc.

Key words: anxiety, etizolam, crossover design, cognitive performance.

INTRODUCTION

Anxiety is an ancient phylogenetic emotion,[1] representing a physiologic reaction to perceived danger. It triggers neural networks that elicit the so-called "fight or flight" response. Anxiety is associated with somatic and autonomic arousal, which varies in intensity and quality.[2] Anxiety disorders include a number of psychiatric conditions in which anxiety causes distressing and often neglected cognitive, behavioral, and somatic symptoms that affect >10% of the population worldwide over the course of their lifetime.[3–5]

Benzodiazepines (BZDs) are effective in the treatment of anxiety.[6] These agents are characterized by an acceptable safety profile when used as directed, but they can cause impairment of memory and other cognitive functions. Adverse events include dose-related anterograde amnesia (ie, forgetfulness of events occurring after drug intake), which may persist for several hours. The intensity of this amnesic effect depends on the $t_{1/2}$, onset of action, and dosage of the BZD. Other major adverse events include sedation and impairment of psychomotor performance.[7] Tolerance and dependency may lead to the misuse or abuse of BZDs.[8] Although appropriate use of these substances should include treatment of a short duration (eg, several weeks), they are often used for >1 year.[9] The exception to short-term use would be therapy for chronic anxiety disorders (eg, general anxiety disorder), which by definition have a duration of ≥6 months and require long-term treatment.[10]

The thienotriazolobenzodiazepine derivative etizolam is an anxiolytic drug with a pharmacologic profile similar to that of the classic BZDs. Results from a neurochemical study suggest that etizolam may have specific intrinsic activity on those subpopulations of γ-aminobutyric acid type A receptors (ie, α1, β2, γ2) that are associated with anxiety.[11] Etizolam has weaker intrinsic affinity for those subunits associated with sedation (α2) and myorelaxation (α5).[12] These properties may characterize etizolam as a ligand with fewer of the adverse events typical of full agonists (impaired cognitive function, tolerance, and dependence). Because of these characteristics, etizolam might be useful in the treatment of chronic anxiety disorders requiring extended treatment, provided that some interruptions are observed and the minimum effective dose is used.[13] Etizolam is approved in Italy for the short-term and long-term treatment of anxiety, in all its manifestations.[14]

The primary objective of the present study was to test for the noninferiority of etizolam 0.5 mg BID compared with placebo in affecting cognitive function in patients with mild to moderate anxiety disorder of recent onset (<1 month). The dosage chosen was that recommended for treating anxiety in Italy.[14,15] The purpose of a noninferiority trial is to determine whether a test treatment is no worse than a reference treatment by more than the equivalence margin.[16–18] The objective here was to determine if the test treatment (etizolam) was at least as good as a reference treatment (placebo) in not modifying cognitive functions. For the goals of the present study, this design was considered statistically robust and more appropriate than an equivalence trial. Secondary objectives included investigating anxiety measures and the tolerability of etizolam 0.5 mg BID in this population.

PATIENTS AND METHODS

Study Patients

To be eligible for the study, patients had to be between 18 and 65 years of age and satisfy the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*)[19] for anxiety disorder not otherwise specified of recent onset (<1 month) and have a Hamilton Anxiety Rating Scale (HAM-A) total score between 16 and 30.[20] This *DSM-IV* category includes disorders with prominent anxiety or phobic avoidance that do not meet criteria for any specific anxiety disorder, adjustment disorder with anxiety, or adjustment disorder with mixed anxiety and depressed mood. According to local ethics committee requirements, a study on healthy volunteers was not feasible for ethical reasons; patients with mild to moderate anxiety disorder were therefore enrolled.

All patients signed an informed-consent form before treatment. The study protocol was approved by the ethics committees of the participating centers and was conducted according to International Conference on Harmonisation (ICH)/Good Clinical Practice guidelines.[21]

Exclusion criteria included any medical (eg, cardiac, liver, renal, endocrinologic, neoplastic, pulmonary) or neurologic disease and other clinical conditions potentially resulting in altered absorption, excessive accumulation, impaired metabolism, or altered excretion of the study medication; any other treatment with antidepressants, anxiolytics, barbiturates, or β-blockers; a history of drug addiction or alcohol abuse (within the last 2 years); participation in another clinical trial within 1 month or having received an investigational drug within the last month before study entry; and known allergic reactions and/or hypersensitivity to any ingredients of the compound. Women of childbearing potential were required to have a negative result on a pregnancy test.

Study Design

This double-blind, placebo-controlled study, performed in 5 centers in Italy, was carried out according to a 2-treatment, 3-period crossover design. Patients were randomized to 3-week sequences of etizolam-placebo-placebo or placebo-etizolam-etizolam. A crossover design was used to reduce the expected intersubject variability and allow a more accurate estimation of changes in cognitive performance. The 2-treatment, 3-period crossover design was also selected for its statistical properties: it allows the most efficient assessment (lower variance) of both the treatment effect (given the carryover effect) and the carryover effect (given the treatment effect).[16,17] Patients were evaluated at 4 scheduled visits (screening and days 7, 14, and 21).

Assessments

Cognitive function, the primary outcome variable, was evaluated using the Wechsler Adult Intelligence Scale (WAIS) Digit Span test (forward and backward digit spans were summed to obtain the score).[22,23] This instrument has been widely used in routine neuropsychological clinical assessments to investigate short-term and working memory and their modulation by fluctuations of attention.[24] The time required to complete the test was recorded and a post hoc analysis was performed by comparing the number of patients who had differences of >5 seconds between periods. A threshold of 5 seconds was used because it approximates the time that elapsed between when the patients received the test material and when they actually started taking the test.

Psychometric evaluation of anxiety was performed using the HAM-A, a 14-item test measuring the severity of anxiety symptoms.[20] Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0 to 56; scores <17 suggest mild anxiety, 18 to 24 mild to moderate anxiety, and 25 to 30 moderate to severe anxiety. The State-Trait Anxiety Inventory (STAI), a 40-question self-administered test for measuring anxiety as a *state* (ie, temporary and situational anxiety) or *trait* (ie, general, long-standing proneness to anxious situations), was also used.[25] The STAI scale has 20 questions each for state and trait anxiety, and each question is scored on a 5-point Likert-type scale from "almost always" to "almost never." HAM-A and STAI were administered at baseline for screening purposes. At each follow-up visit, patients were evaluated using the HAM-A and STAI (state) tools.

To assess somatic tolerability, vital signs (blood pressure and heart rate), weight, and adverse events were recorded at each visit. Patients underwent a physical examination in which vital signs were measured in the sitting position, after which adverse events were recorded in the context of an unstructured clinical interview. The consensus among the authors regarding what should constitute a significant change in body weight was that a 10% change in either direction should be considered.

Compliance was assessed by tablet counting at the start of the study and at each follow-up visit. All evaluations were performed by a psychiatric resident who was under the supervision of a senior psychiatrist (M.P.D., G.D., F.D., C.M., M.R., E.A., A.G., G.B., A.D., and G.P.) in the outpatient facilities of the psychiatric departments involved in the study.

Statistical Analysis

Descriptive statistics were calculated for qualitative (absolute and percentage frequencies) and quantitative (arithmetic mean, SD, minimum, and maximum) variables.

The carryover effect was tested by ANCOVA for a linear model of a 2-treatment and 3-period crossover

design, with the basal value as covariate.[26] The same model was used for testing the statistical hypothesis on the secondary objective (tolerability) concerning blood pressure, heart rate, and weight, as well as for evaluation of the anxiety rating scales (HAM-A and STAI [state]).

The significance of differences between etizolam and placebo was evaluated using the CI approach on the difference between the 2 treatments calculated within the same subjects; this difference was obtained considering the values of the last 2 periods while taking placebo versus the value at the end of the first period with etizolam in the etizolam-placebo-placebo sequence and likewise for the placebo-etizolam-etizolam sequence. If there was an improvement of the target variable while receiving placebo, the noninferiority hypothesis of etizolam versus placebo would be rejected if the upper 95% confidence limit of the CI for the difference between the change with placebo and with etizolam was more than the noninferiority threshold; otherwise, in the case of a worsening of the target variable while receiving placebo, the noninferiority hypothesis of etizolam versus placebo would be rejected if the lower 95% confidence limit of the CI was lower than the noninferiority threshold.

The initial sample size of ~80 patients, allocated to the treatment sequences, allowed for a power of at least 0.80 to test a noninferiority hypothesis, with the maximal allowable difference being ~32% of the phenomenon variability and with a statistical significance of 0.05 (2-tailed). The variability of the phenomenon was obtained from the literature as a value of 2.5 to 3 points[22] and, consequently, the maximum allowable difference for claiming the noninferiority of etizolam versus placebo was set at 0.75 in the case of an improvement of the target variable or at –0.75 in the case of a worsening of a target variable. The mean change while receiving etizolam could not be more than 0.75 compared with the mean change while receiving placebo; this threshold is consistent with <1 point of the WAIS Digit Span test. In addition, following a conservative approach, it was planned that the hypothesis also be tested with the noninferiority threshold recalculated using the lowest value of the phenomenon variability obtained from the actual study data.

The prevalence of adverse events was compared between the 2 regimens using the Fisher exact test. Cardiovascular function and weight were considered as the percentage change from the previous recorded value. An *adverse event* was defined as an absolute change >20% for heart rate and systolic or diastolic blood pressure, and >10% for weight. The cumulative prevalence of the occurrence of these events while receiving etizolam or placebo was compared using the Fisher exact test.

The analysis was conducted on the intent-to-treat (ITT) population (ie, all patients randomized to receive treatment). However, according to the ICH E9 guideline ("Statistical Principles for Clinical Trials"),[27] using the full analysis set is generally not a conservative approach; indeed, use of a last-observation-carried-forward approach can lead to a lower estimate of the actual effect of an active drug on cognitive functions. Therefore, a "restricted" ITT population was also analyzed, which comprised those patients who took at least 1 dose of both sequences, with missing data input according to the last observation carried forward for those patients who withdrew from the trial just before the last visit.

The statistical analysis was conducted using SAS version 8.2 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Study Patients

Baseline demographic and clinical characteristics of the ITT population (ie, all patients randomized to receive treatment [N = 77]) are shown in **Table I**. There were no significant differences between the 2 treatment sequence groups for all the variables analyzed. A total of 77 white patients were enrolled (mean age, 33.3 years [range, 22–60 years]; 62.3% female; mean weight, 65.2 kg). Six patients withdrew from the trial after the screening visit but before receiving the first dose of treatment: 5 of these patients withdrew their consent (3 patients randomized to the etizolam-placebo-placebo sequence and 2 randomized to the placebo-etizolam-etizolam sequence) and 1 patient (randomized to the etizolam-placebo-placebo sequence) was withdrawn because of lack of compliance.

Two other patients (randomized to the etizolam-placebo-placebo sequence) withdrew before the last visit (citing "lack of efficacy" and "adverse event" [headache of moderate intensity], respectively, both while receiving placebo), but they were included in the restricted ITT analysis (n = 71). The main statistical analysis, however, was conducted on the entire randomized population of 77 patients.

Table I. Baseline demographic and clinical characteristics* of the intent-to-treat population (N = 77) according to treatment sequence.

Characteristic	Treatment Sequence: Etizolam-Placebo-Placebo (n = 39)	Treatment Sequence: Placebo-Etizolam-Etizolam (n = 38)	Total (N = 77)
Age, y	33.5 (8.26)	33.1 (9.21)	33.3 (8.68)
Sex			
Female	22 (56.4)	26 (68.4)	48 (62.3)
Male	17 (43.6)	12 (31.6)	29 (37.7)
Weight, kg	66.6 (13.8)	63.8 (11.0)	65.2 (12.5)
WAIS Digit Span†	8.41 (1.86)	8.47 (1.98)	8.44 (1.91)
WAIS Digit Span time, sec	21.51 (8.37)	23.08 (16.77)	22.29 (13.13)
HAM-A‡	22.5 (4.08)	21.9 (3.21)	22.2 (3.66)
STAI (state)§	50.6 (13.79)	53.7 (13.63)	52.2 (13.71)
STAI (trait)‖	47.0 (10.83)	49.4 (12.09)	48.2 (11.45)
Blood pressure, mm Hg			
Systolic	118.3 (7.72)	115.4 (8.96)	116.9 (8.43)
Diastolic	76.2 (7.73)	73.9 (8.31)	75.1 (8.05)
Heart rate, beats/min	73.9 (8.47)	73.9 (8.09)	73.9 (8.23)

WAIS = Wechsler Adult Intelligence Scale; HAM-A = Hamilton Anxiety Rating Scale; STAI = State-Trait Anxiety Inventory.
*Data are given as absolute and percentage frequencies for sex; the rest are mean (SD).
† Combined raw scores from the forward and backward assessments.
‡ Based on a scale of 0 (not present) to 4 (severe), with a total score range of 0 to 56. Scores <17 suggest mild anxiety, 18 to 24 mild to moderate anxiety, and 25 to 30 moderate to severe anxiety.
§ Based on 20 items pertaining to state anxiety (ie, temporary and situational anxiety), each scored on a 5-point scale.
‖ Based on 20 items pertaining to trait anxiety (ie, general, long-standing proneness to anxious situations), each scored on a 5-point scale.

The intensity of anxiety disorder, as measured using the HAM-A, was mild in 34 patients (44.2%), moderate in 31 patients (40.3%), and intermediate (between mild and moderate) in 12 patients (15.6%).

Cognitive Function

The mean (SD) values of the WAIS Digit Span scores (forward and backward) at each visit are shown in **Table II**. The mean score for all patients, independent of treatment sequence, showed an increase from baseline of 3.9% on day 7, followed by an increase of 13.7% on day 14 and 12.0% on day 21.

Mean values on the WAIS Digit Span test decreased after the first period while receiving etizolam (0.36%); they increased after the first period while receiving placebo (8.14%). In the etizolam-placebo-placebo sequence, there was an increase at the end of each of the 2 periods while receiving placebo (12.3% and 10.7%, respectively) compared with the end of the first period while receiving etizolam. Similarly, in the placebo-etizolam-etizolam sequence, after the initial increase at the end of the first period with placebo, a further increase occurred at the end of the last 2 periods with etizolam (6.88% and 5.13%, respectively, compared with the end of the first period with placebo).

With a power of 0.80, the difference between the effects of etizolam and placebo on WAIS Digit Span score performance was not significant for total score (0.102 [90% CI, –0.130 to 0.335]). The 90% CI included the null value (no statistically significant differ-

Table II. Mean (SD) scores on the Wechsler Adult Intelligence Scale Digit Span test according to treatment sequence in the intent-to-treat population (N = 77).*

	Treatment Sequence		
Time Point	Etizolam-Placebo-Placebo (n = 39)	Placebo-Etizolam-Etizolam (n = 38)	Total (N = 77)
Screening	8.41 (1.86)	8.47 (1.98)	8.44 (1.91)
Day 7	8.38 (1.77)	9.16 (1.46)	8.77 (1.66)
Day 14	9.41 (1.98)	9.79 (1.79)	9.60 (1.87)
Day 21	9.28 (1.95)	9.63 (1.73)	9.45 (1.84)

*Combined raw scores from the forward and backward assessments.

ence between the 2 regimens). Of more relevance according to the noninferiority hypothesis, the upper limit of 0.335, which was consistent with the unilateral confidence coefficient of 0.95, was lower than the noninferiority threshold of 0.75. This 95% unilateral upper confidence limit was also lower than the noninferiority threshold of 0.48 obtained using the lowest value of the variability from the study data. Similar results were obtained using the more conservative restricted ITT population (n = 71), where the difference was 0.122 (90% CI, –0.125 to 0.369).

Table III shows the mean (SD) values at each visit for the time required to complete the WAIS Digit Span test. The mean of all patients revealed an increase of 2.69% from baseline on day 7, a decrease of 6.33% from baseline on day 14, and an additional decrease of 10.41% from baseline on day 21. Mean values increased after the first period with etizolam (4.28%) and with placebo (2.08%). In the etizolam-placebo-placebo sequence, mean values decreased (after an initial increase in the first period with etizolam) at the end of the 2 subsequent periods with placebo (8.43% and 13.02%, respectively). Similarly, in the placebo-etizolam-etizolam sequence, mean values decreased (after an increase in the first period with placebo) at the end of the 2 subsequent periods with etizolam (9.85% and 13.20%, respectively).

Hence, the difference between the effects of etizolam and placebo on time required for completion of the WAIS Digit Span test was not significant (0.029 second [90% CI, –0.574 to 0.632]). The 90% CI included the null value (no statistically significant difference between the 2 treatments) and the upper limit of 0.632 (consistent with a unilateral confidence coefficient of 95%), which was below the noninferiority threshold of 0.735 derived from the actual study data using the ANCOVA model. Similar results were obtained with the more conservative restricted ITT

Table III. Time (in seconds [mean (SD)]) required to complete the Wechsler Adult Intelligence Scale Digit Span test according to treatment sequence in the intent-to-treat population (N = 77).

	Treatment Sequence		
Time Point	Etizolam-Placebo-Placebo (n = 39)	Placebo-Etizolam-Etizolam (n = 38)	Total (N = 77)
Screening	21.51 (8.37)	23.08 (16.77)	22.29 (13.13)
Day 7	22.43 (10.59)	23.56 (16.62)	22.89 (13.81)
Day 14	20.54 (9.08)	21.24 (16.91)	20.88 (13.44)
Day 21	19.51 (8.65)	20.45 (17.05)	19.97 (13.39)

population, where the difference between the change while receiving placebo and the change while receiving etizolam was –0.005 (90% CI, –0.634 to 0.643).

Only 16 patients (20.8%) reported a change of >5 seconds in the time required to complete the WAIS Digit Span test when comparing between periods. This occurred in 6 patients while receiving etizolam and in 6 patients while receiving placebo; 4 patients reported an increase during both etizolam and placebo administration.

Anxiety Assessment

Independently from treatment sequence, patients reported an overall decrease in mean total HAM-A score from baseline (22.2 [3.66]) of 10.8% at day 7 (19.8 [4.45]), 23.0% at day 14 (17.1 [5.29]), and 32.9% at day 21 (14.9 [5.80]). Mean values decreased after the first period while receiving etizolam (baseline: 22.5 [4.08] to 20.0 [5.00], 11.1%) or while receiving placebo (baseline: 21.9 [3.21] to 19.5 [3.87], 10.9%). Furthermore, in the etizolam-placebo-placebo sequence, mean values decreased by 22.5% at the end of the last 2 periods while receiving placebo (day 14, 17.4 [5.42]; day 21, 15.5 [5.92]). Similarly, in the placebo-etizolam-etizolam sequence, mean values decreased by 26.7% at the end of the last 2 periods with etizolam (day 14, 16.8 [5.23]; day 21, 14.3 [5.69]).

From the ANCOVA analysis, no significant carryover effect was detected. The difference between the 3 periods was statistically significant ($P < 0.001$), and the greater decrease with etizolam treatment was not statistically significant. There was no statistically significant relationship between baseline values and periods. Similar results were obtained from the restricted ITT population of 71 patients.

Considering the STAI (state) total score for all patients independently of treatment sequence, there was a decrease from baseline (52.2 [13.71]) of 8.2% on day 7 (47.9 [11.61]), 15.5% on day 14 (44.1 [10.44]), and 19.7% on day 21 (41.9 [10.67]). Although the carryover effect was not statistically significant based on the ANCOVA analysis, there was a significant difference between the 3 periods considered ($P < 0.001$) and a statistically significant relationship between baseline value and the other treatment periods ($P < 0.001$). Furthermore, the difference between the 2 regimens was at the limit of significance ($P < 0.052$). Patients receiving etizolam exhibited a greater decrease than those receiving placebo. The decrease was 8.9% while receiving etizolam in the first period of the etizolam-placebo-placebo sequence (from 50.6 [13.79] to 46.1 [11.53]) and 14.8% at the end of the last 2 periods with etizolam in the placebo-etizolam-etizolam sequence (from 49.9 [11.53] at day 7 to 44.4 [10.58] at day 14 and to 42.6 [10.76] at day 21). While receiving placebo, there was a decrease of 7.1% in the first period of the placebo-etizolam-etizolam sequence (from 53.7 [13.63] to 49.9 [11.54]) and 10.4% at the end of the last 2 periods with placebo in the etizolam-placebo-placebo sequence (from 46.1 [11.53] at day 7 to 43.8 [10.44] at day 14 and to 41.3 [10.68] at day 21). Similar results were obtained from the restricted ITT population of 71 patients.

Compliance, as assessed by drug accountability (number of returned pills), was >95% in the restricted ITT population of 71 patients who received treatment (the 6 patients who underwent only the screening visit did not receive medication).

Tolerability

Using the ANCOVA model, no statistically significant difference was found on the carryover effect, the differences between periods, or the direct treatment effect for any of the investigated variables.

The frequency of cardiovascular events while receiving etizolam 0.5 mg BID was not statistically different from that while receiving placebo. One patient had an increase in systolic blood pressure >20% while receiving etizolam; it returned to the baseline value by study end. There were 4 minor events involving diastolic blood pressure; these occurred in 2 patients receiving etizolam and 2 patients receiving placebo. Four minor events regarding heart rate were observed in 2 patients receiving etizolam and 2 patients receiving placebo. However, all blood pressure and heart rate values remained within normal ranges.

One patient had an increase in weight of >10% in the 2 weeks after receiving placebo. The difference between the 2 regimens was nonsignificant.

Twelve of the 77 patients (15.6%) reported adverse events (the difference between the 2 regimens was not significant). Four patients experienced moderate headache, 2 patients had mild nausea, and 1 patient had a mild "bruise on the legs" only during the period while receiving placebo; 3 patients reported somnolence (mild in 2 patients and moderate in 1 patient) and 1 patient reported moderate weakness only during the period while receiving etizolam. Four patients report-

ed mild somnolence while receiving etizolam and while receiving placebo.

DISCUSSION

The lack of statistically significant carryover effect for all analyzed variables confirmed the validity of the crossover design in the present study. No significant differences between treatments were found in the WAIS Digit Span scores, including when conservative calculations based on the actual variability observed in the study were made. No significant differences between etizolam 0.5 mg BID and placebo were found regarding the time required to perform the WAIS Digit Span test.

None of the patients had an increase in anxiety as assessed by the HAM-A and STAI (state) scores; scores on the rating scales did not decrease significantly over the 3 weeks of treatment. The nature of the disorder and the individual psychological support provided may account for the nonsignificant decrease in scores on the anxiety rating scales, particularly those of the HAM-A, during the placebo periods of the crossover trial.

There were no significant changes in blood pressure, heart rate, or weight with either etizolam or placebo. Adverse events were not statistically different with etizolam or placebo. A possibly relevant adverse event—mild or moderate somnolence—was reported by 7 of 77 patients (9.1%). Four of these patients reported somnolence both with etizolam and with placebo. Noteworthy, the findings on attention and memory are consistent with the pharmacologic profile of etizolam as a partial agonist.[11]

The duration of this study was considered sufficient for the purpose of assessing cognitive function in patients with anxiety disorder not otherwise specified, and the crossover design supports the validity of the clinical results of the comparison.

Limitations

Limitations of this study include the lack of an active comparator, which would have helped to confirm that the absence of a significant difference between treatments was not due to lack of sensitivity or power. However, the sensitivity of the study is that a 1-point change on the WAIS Digit Span test could be detected with a probability of 0.80.

Another limitation of the study may be the seemingly low dosage of etizolam (0.5 mg BID), which could have masked toxic effects and accounted for the absence of the cognitive dysfunctions caused by BZDs. However, 0.5 mg BID is the mean effective dosage suggested by the Italian Drug Regulatory Agency, and it is the mean dosage administered by Italian psychiatrists and general practitioners to patients with anxiety disorders.[14] The dosage used in the study is therefore clinically relevant, based on current prescription guidelines. The observed lack of difference in clinical response may be due to other, unanalyzed variables. The intense clinical attention resulting from the study design may itself have caused a placebo effect that masked differences in clinical response between treatments.[15]

The last limitation of this study is the sample enrolled, which was a group of young white patients with anxiety of mild to moderate severity and of recent onset. This could limit the ability to extrapolate the results to other populations. The majority of patients with anxiety who visit general practitioners in Italy, however, are represented by this group of patients, making our sample reasonably characteristic of a large portion of the BZD-treated population with anxiety.[28]

CONCLUSIONS

Neuropsychological assessment did not detect any significant differences between etizolam 0.5 mg BID and placebo in cognitive function or anxiety measures in this study of patients with anxiety. Etizolam was well tolerated.

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REFERENCES

1. Denver RJ. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Ann N Y Acad Sci*. 2009;1163:1–16.
2. Bersani G. *Le Benzodiazepine Nella Pratica Medica e Psichiatrica*. Rome, Italy: Giovanni Fioriti Editore; 2001.

3. Tiemens BG, Ormel J, Simon GE. Occurrence, recognition, and outcome of psychological disorders in primary care. *Am J Psychiatry*. 1996; 153:636–644.
4. Bijl RV, Ravelli A, van Zessen G. Prevalence of psychiatric disorder in the general population: Results of The Netherlands Mental Health Survey and Incidence Study (NEMESIS). *Soc Psychiatry Psychiatr Epidemiol*. 1998; 33:587–595.
5. Kessler RC, Berglund P, Demler O, et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication [published correction appears in *Arch Gen Psychiatry*. 2005;62:7658]. *Arch Gen Psychiatry*. 2005;62:593–602.
6. Davidson JR. First-line pharmacotherapy approaches for generalized anxiety disorder. *J Clin Psychiatry*. 2009;70(Suppl 2):25–31.
7. Longo LP, Johnson B. Addiction: Part I. Benzodiazepines—side effects, abuse risk and alternatives. *Am Fam Physician*. 2000;61:2121–2128.
8. Licata SC, Rowlett JK. Abuse and dependence liability of benzodiazepine-type drugs: GABA(A) receptor modulation and beyond. *Pharmacol Biochem Behav*. 2008;90:74–89.
9. Neutel CI. The epidemiology of long-term benzodiazepine use. *Int Rev Psychiatry*. 2005;17:189–197.
10. Lydiard RB. An overview of generalized anxiety disorder: Disease state—appropriate therapy. *Clin Ther*. 2000; 22(Suppl A):A3–A19; discussion A20–A24.
11. Sanna E, Pau D, Tuveri F, Massa F, et al. Molecular and neurochemical evaluation of the effects of etizolam on GABAA receptors under normal and stress conditions. *Arzneimittelforschung*. 1999;49:88–95.
12. Biggio G, Sanna E, Serra M. Etizolam, un ansiolitico con ridotta capacità di indurre tolleranza e sedazione. Basi molecolari. *Edit Symposia Psichiatrica, Speciale Biologia Molecolare*. Ferrara, Italy: Editeam; 2004.
13. Sanna E, Busonero F, Talani G, et al. Low tolerance and dependence liabilities of etizolam: Molecular, functional, and pharmacological correlates. *Eur J Pharmacol*. 2005; 519:31–42.
14. Information on etizolam at the Torrinomedica Institute. http://www.torrinomedica.it/studio/generaframe.asp?variabile=http://www.torrinomedica.it/farmaci/schedetecniche/PASADEN.asp. Accessed November 26, 2009.
15. Bellantuono C, Balestrieri M. *Gli Psicofarmaci*. Rome, Italy: Pensiero Scientifico Editore; 1997.
16. D'Agostino RB Sr, Massaro JM, Sullivan LM. Non-inferiority trials: Design concepts and issues—the encounters of academic consultants in statistics. *Stat Med*. 2003;22:169–186.
17. Snapinn SM. Noninferiority trials. *Curr Control Trials Cardiovasc Med*. 2000;1:19–21.
18. Piaggio G, Elbourne DR, Altman DG, et al, for the CONSORT Group. Reporting of noninferiority and equivalence randomized trials: An extension of the CONSORT statement [published correction appears in *JAMA*. 2006;296:1842]. *JAMA*. 2006;295:1152–1160.
19. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*. 4th ed. Washington, DC: American Psychiatric Association; 1994.
20. Bruss GS, Gruenberg AM, Goldstein RD, Barber JP. Hamilton Anxiety Rating Scale Interview guide: Joint interview and test-retest methods for interrater reliability. *Psychiatry Res*. 1994;53:191–202.
21. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Table of contents. http://ichgcp.net/. Accessed September 18, 2009.
22. Wilde NJ, Strauss E, Tulsky DS. Memory span on the Wechsler scales. *J Clin Exp Neuropsychol*. 2004; 26:539–549.
23. Wechsler D. *WMS-R: Wechsler Memory Scale—Revised Manual*. San Antonio, Tex: The Psychological Corporation, Harcourt Brace Jovanovich; 1987.
24. Orsini A, Grossi D, Capitani E, et al. Verbal and spatial immediate memory span: Normative data from 1355 adults and 1112 children. *Ital J Neurol Sci*. 1987;8:539–548.
25. Spielberger CD, Gorsuch RL, Lushene RE. *Manual for the State-Trait Anxiety Inventory*. Palo Alto, Calif: Consulting Psychologists Press; 1970.
26. Jones B, Kenward MG. *Design and Analysis of Cross-Over Trials*. New York, NY: Chapman and Hall; 1989.
27. European Medicines Agency. ICH E9, Statistical Principles for Clinical Trials. http://www.emea.europa.eu/pdfs/human/ich/036396en.pdf. Accessed January 30, 2009.
28. Blashki G, Judd F, Piterman L, Fumagalli E. Psichiatria e medicina generale. Milan, Italy: McGraw-Hill; 2007.

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