Alzheimer Disease and Associated Disorders
Vol. 10, No. 2, pp. 93–102

A Treatment and Withdrawal Trial of Besipirdine in Alzheimer Disease

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Summary: Besipirdine hydrochloride (HP 749) is an indole-substituted analog of 4-aminopyridine. Besipirdine enhances both cholinergic and adrenergic neurotransmission in the central nervous system. The present study examined the efficacy and tolerability of two doses of besipirdine (5 and 20 mg b.i.d.) in 275 patients with Alzheimer disease during 3 months of treatment and for 3 months after withdrawal of treatment. Assessment after withdrawal of treatment was used in an effort to distinguish persistent efficacy attributable to a neuroprotective mechanism from reversible symptomatic efficacy. Besipirdine was generally well tolerated. The level of performance on the cognitive subscale of the Alzheimer Disease Assessment Scale (ADAS-Cog) was sustained during 3 months of treatment with besipirdine, whereas some deterioration in the performance of patients treated with placebo was observed over the same period. The small difference between active and placebo treatment groups approached, but did not reach statistical significance in the primary intent-to-treat analysis ($p = 0.067$); analysis of patients who completed all assessments was supportive ($p = 0.031$). Global ratings using the Clinician Interview-Based Impression of Change did not detect a besipirdine treatment benefit, possibly because of an adverse effect on mood and behavior in some patients. A high ratio of adrenergic to cholinergic potency may have resulted in the adverse effects of besipirdine and hence its failure to support the hypothesis that multiple neurotransmitter treatment may be more efficacious than monotherapy. The efficacy apparent on the ADAS-Cog after 3 months of treatment did not persist 3 months after withdrawal of treatment, suggesting that the benefit was symptomatic. This study provides a practical example of the use of treatment withdrawal assessment to distinguish neuroprotective from symptomatic efficacy. **Key Words:** Cholinergic—Adrenergic—Pharmacology—Besipirdine—Treatment withdrawal.

Neurochemical studies comparing postmortem brain specimens from patients with Alzheimer disease (AD) against specimens from age-matched nondemented individuals have revealed deficiencies in several of the classical neurotransmitters. A deficiency of acetylcholine is the most prominent in terms of the average magnitude across AD cases, the consistency of the finding from one study to another, and the correlation with dementia symptoms (Davies and Maloney, 1976; Perry et al., 1978; Terry and Katzman, 1983). Related to the loss of acetylcholine in the cerebral cortex is a loss of cholinergic cells in the nucleus basalis of Meynert, which projects to the cortex (Whitehouse et al., 1982). Probably next in importance behind acetylcholine among neurotransmitters that are

Received July 28, 1995. Revised manuscript accepted January 10, 1996.
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deficient in AD is norepinephrine, which has been found to be depleted in cerebral cortex in numerous studies (Adolfsson et al., 1979; Perry et al., 1981; Mann et al., 1982; Arai et al., 1984; Rossor and Iversen, 1986). A corresponding loss of noradrenergic neurons has been documented in the locus ceruleus, which projects to the cerebral cortex (Bondareff et al., 1982).

Treatment of AD has been attempted using strategies that augment cholinergic activity in the central nervous system. The most successful have been acetylcholinesterase inhibitors: tacrine (Davis et al., 1992; Farlow et al., 1992; Knapp et al., 1994) and velnacrine (Antuono, 1995) have produced symptomatic benefits measured by the Alzheimer Disease Assessment Scale (Rosen et al., 1984) and by clinician-rated global evaluations. Evidence that activation of muscarinic receptors modifies the processing of amyloid precursor protein (Nitsch et al., 1992) suggests that cholinergic treatments may affect the progression of the disease through a neuroprotective mechanism, although this hypothesis is yet to be proved clinically.

The adrenergic agent clonidine has been shown to improve memory deficits in adrenergic-deficient monkeys (Arnsten and Goldman-Rakic, 1985). Efficacy was strongly dose dependent, however, consistent with the pharmacology of clonidine. At low doses clonidine inhibits presynaptic release of norepinephrine, whereas at higher doses it stimulates postsynaptic alpha-2 receptors. A study of clonidine as a treatment for AD (Mohr et al., 1989) found no consistent effect of clonidine on attention, verbal memory, or visual-spatial memory. The doses used were possibly too low to achieve cognitive effects, however, and dose escalation was limited by the emergence of adverse effects, including symptomatic hypotension and mild sedation.

Simultaneous treatment of both the cholinergic and adrenergic deficits in AD may be more efficacious than monotherapy for either deficit alone. Initial efforts at combined cholinergic and adrenergic treatment (reviewed in Huff, 1995) have been unsuccessful owing to the emergence of adverse effects or equivocal efficacy results. Potassium channel blockers have been examined as potential cholinergic and adrenergic treatments for AD. The cholinesterase inhibitor tacrine blocks potassium channels at high concentrations (Drukarch et al., 1987). The nonselective potassium channel blocker 4-aminopyridine is known to increase the release of acetylcholine and possibly norepinephrine at brain synapses (Lavretsky and Jarvik, 1992).

Besipirdine (HP 749) is an indole-substituted analog of 4-aminopyridine (Cornfeldt et al., 1990; Huger et al., 1990; Hubbard et al., 1991). Besipirdine selectively blocks the M-channel, a potassium channel that is inactivated by muscarinic agonists. However, besipirdine is not a potent muscarinic or nicotinic agonist. There is evidence of an independent binding site for besipirdine, which may mediate the M-channel effect. Besipirdine is not an effective acetylcholine releaser: basal release of acetylcholine occurs only at high (millimolar) concentrations. It also is not a cholinesterase inhibitor.

Besipirdine reverses scopolamine-induced memory deficit on passive avoidance memory in mice. Across the dose range 0.02–0.31 mg/kg, the optimal dose of besipirdine is 0.08 mg/kg in this paradigm. Besipirdine inhibits the neuronal presynaptic uptake of the monoamines norepinephrine, dopamine, and, to a lesser extent, serotonin. It is also an alpha-2 receptor antagonist that increases release of norepinephrine from presynaptic terminals. Besipirdine is extensively metabolized and conjugated to form glucuronides. The N-dealkylated principal metabolite of besipirdine is an alpha-1 agonist and alpha-2 antagonist. In rats with lesions in both the cholinergic nucleus basalis and the adrenergic ascending noradrenergic bundle, besipirdine reversed a deficit in retention of passive dark avoidance at doses ranging from 0.02 to 0.08 mg/kg (Santucci et al., 1991). The 0.08 mg/kg dose corresponds to a human dose of ~5 mg. In healthy elderly volunteers, the plasma half-life of a single 20-mg dose was 12.6 h.

Tolerability of besipirdine in patients with AD has been examined in ascending doses of 5–60 mg b.i.d. (Sramek et al., 1995). The minimum dose for an intolerable adverse effect was 60 mg b.i.d., at which dose one of eight patients experienced angina. The angina resolved within hours, and a myocardial infarction was excluded by follow-up examinations. Angina is attributable to the alpha-1 agonist vasoconstrictive property of the N-dealkylated metabolite of besipirdine. The dose range selected for efficacy studies was therefore 5–40 mg b.i.d. Adverse effects seen in this dose range include bradycardia and postural hypotension, which are attributable to both cholinergic and adrenergic effects.

The efficacy and tolerability of besipirdine at doses of 5 mg b.i.d. and 20 mg b.i.d. during 3 months of treatment and a subsequent 3-month treatment-withdrawal observation period were investigated in the present study, which provides the first controlled information regarding the efficacy of besipirdine in patients with AD.

METHODS

Patients

Eligible patients were men and women of any race who met the diagnostic criteria for probable AD estab-

lished by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984) whose cognitive deficits had been present for ~1 year, with mild to moderate dementia at study entry as defined by Mini-Mental State Examination scores of 10–28 (Folstein et al., 1975; Bleeker et al., 1988) and by Global Deterioration Scale ratings ≥3 (Reisberg et al., 1982). Patients were excluded who were likely to have vascular dementia, defined by scores of ≥5 on the modified Hachinski Ischemia Scale (Rosen et al., 1979), or who had other prospectively identified neurologic, psychiatric, or medical conditions documented by history, examination, or diagnostic tests, including brain imaging.

Concurrent use of other investigational compounds or drugs known to produce bradycardia was prohibited. Psychotropic medications were limited to intermittent use of haloperidol and hydroxyzine, which were required to be withheld for 3 days before efficacy assessments. Informed consent was required from caregivers and patients or their legal representatives.

Study Design

The study comprised two 12-week, double-blind, parallel-group, placebo-controlled treatment segments, followed by an open-label extended-treatment segment. One-third of patients were randomly assigned to each of three Segment I treatment groups: 5 mg b.i.d. besipirdine, 20 mg b.i.d. besipirdine, and placebo b.i.d. (Fig. 1). Patients assigned to the 20 mg b.i.d. besipirdine group received 5 mg b.i.d. for 2 weeks and then 10 mg b.i.d. for 2 weeks, as a titration to 20 mg b.i.d. At study entry, one of every 12 patients assigned to active treatment in Segment I was assigned randomly to continue active treatment during Segment II, in order to maintain blinding of treatment assignments. All other patients received placebo in Segment II. Patients who completed Segment II were eligible to enter the open-label treatment segment.

The purpose of Segment II was to differentiate potential neuroprotective and symptomatic effects of besipirdine. This assessment was based on the following assumptions and definitions.

(a) Neuronal degeneration and dementia in Alzheimer disease proceed at a roughly constant rate in the absence of treatment. Although the rate of disease progression varies among patients and may change during the course of the disease, this assumption is appropriate for comparison of mean changes between groups that are equivalent with regard to disease severity at the initiation of treatment.

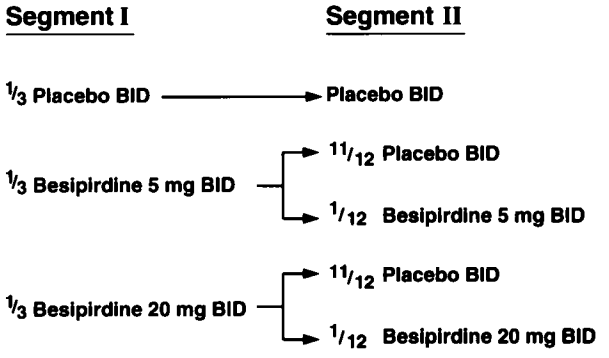

FIG. 1. Schematic summary of randomization into treatment groups (see Study Design). Treatment assignments for Segments I and II were determined simultaneously at study entry.

(b) Neuroprotective treatments slow the rate of disease progression. An optimally effective treatment will arrest disease progression.

(c) A treatment that slows, but does not arrest neuronal degeneration will produce cumulative neuronal sparing over time. The magnitude of treatment effect in comparison to a parallel group of untreated patients will therefore be proportional to the duration of treatment.

(d) After withdrawal of a neuroprotective treatment, disease progression will resume at the constant rate that preceded treatment. Treatment will not permanently change the rate of disease progression if it is withdrawn, and efficacy that arises during treatment owing to cumulative neuronal sparing will persist after withdrawal of treatment. A group of treated patients whose disease has become less severe than that in a parallel untreated group will therefore remain less impaired than the untreated group after withdrawal of treatment, although both groups will progress in disease severity at the same rate after cessation of treatment.

(e) Symptomatic treatments enhance the function of remaining neurons and produce improvements in disease symptoms rather than affecting disease progression. Symptomatic effects that arise gradually after initiation of treatment may appear to slow disease progression because compensatory enhancement of remaining neurons is superimposed upon continued neuronal degeneration, resulting in a net reduction in apparent progression of dementia. However, in contrast to neuroprotective effects, symptomatic effects do not result from neuronal sparing and therefore do not persist after withdrawal of treatment: a previously treated group will soon become indistinguishable from a parallel group of untreated patients. Symptomatic effects may thus be differentiated from neuroprotective effects based on assessment after treatment withdrawal.

(f) An agent may have both symptomatic and neuroprotective effects if it both enhances residual neuronal function and slows or arrests neuronal degeneration. After withdrawal of treatment with such an agent, a fraction of its efficacy will persist, corresponding to its neuroprotective effect. The persistent efficacy must be of sufficient magnitude to be detectable statistically in order to confirm that an agent has mixed symptomatic and neuroprotective effects.

Outcome Measures

The primary efficacy outcome measures specified prospectively by protocol were the Alzheimer Disease Assessment Scale cognitive subscale (ADAS-Cog) (Rosen et al., 1984), which is a psychometric assessment of recent memory, orientation, language, and praxis, and the Clinician Interview-Based Impression of Change (Knopman et al., 1994), which was modified to allow the clinician to interview the caregiver as well as the patient before rendering a rating of global evaluation of change in the patient's dementia symptoms (CIBIC-Plus). After the pretreatment baseline assessment, the ADAS tester and CIBIC-Plus rater were blind to each other's results and to all other clinical and research information regarding the patient. Study personnel were instructed in the performance of the ADAS-Cog and CIBIC-Plus during a prestudy meeting of all investigators and their staff and during individual study-site initiation visits by the monitoring staff. Sites were monitored at least twice during the conduct of the study.

Secondary outcome measures were the ADAS noncognitive subscale (ADAS-Noncog), the Information-Memory-Concentration (IMC) Test and the Dementia Score of the Blessed Dementia Scale (BDS) (Blessed et al., 1968), and the Cornell Scale for Depression in Dementia (Alexopoulos et al., 1988). After the pretreatment baseline, all primary and secondary outcome measures were repeated every 4 weeks throughout Segments I and II except the BDS, which was repeated only at the end of Segment II. Blood samples for determination of besipirdine plasma levels were drawn at the study visits during which outcome measures were assessed. Outcome data regarding caregivers of patients were also collected through the Caregiving Activities Time Survey (Moore and Clipp, 1994) and the Caregiving Hassles Scale (Kinney and Stephen, 1989). Results of plasma levels and the caregiver measures are not presented in this article.

Safety assessments were performed at baseline, every 2 weeks during Segment I, and every 4 weeks during Segment II. Assessments included laboratory blood tests, urinalysis, electrocardiograms, vital signs, and a treatment-emergent symptom checklist. Physical examinations were repeated at the end of Segments I and II.

Statistical Methods

A sample of 261 analyzable patients was calculated to be required in order to provide 80% probability at a 5% alpha level of significance of detecting a two-point difference after 3 months of treatment between the active and placebo groups in ADAS-Cog change scores from baseline. The rate of deterioration on the ADAS-Cog in patients with AD varies with dementia severity (Stern et al., 1994). The patients enrolled in the present study were expected to deteriorate 8 points on average in 12 months, or about 2 points in 3 months, and a treatment effect of similar magnitude is considered clinically important.

The primary study hypothesis was that treatment with besipirdine would favorably affect the core signs and symptoms of AD, as assessed by the ADAS-Cog and supported by the CIBIC-Plus, during 12 weeks of treatment (Segment I). A secondary hypothesis, that besipirdine has a persistent effect after discontinuation of treatment, was tested by computing the change between the pretreatment baseline and the end of Segment II and by testing the prediction that the group that had received besipirdine during Segment I would manifest less deterioration at the end of Segment II than the group that had received placebo during Segment I. If a difference at the end of Segment I were to persist to the end of Segment II, an effect on dementia progression attributable to a neuroprotective mechanism would be inferred.

Two imputation schemes were defined prospectively to address the issue of missing data. The primary imputation scheme was an intent-to-treat analysis using the last observation carried forward for end-point analysis of incomplete cases. The second imputation scheme included only "completers," patients who completed all scheduled assessments of a variable during the study segment under analysis.

The primary analysis combined the active treatment groups (5 mg b.i.d. and 20 mg b.i.d.) in comparison to the placebo treatment group. Each active treatment was compared with placebo separately in secondary analyses. Separate analyses were performed for Segments I and II. Analyses of the ADAS-Cog, ADAS-Noncog, BDS, and Cornell scale were two-tailed tests using general linear models, including terms for treatment group and study center. Two-tailed Mantel-Haenszel tests were used to analyze the CIBIC-Plus data. An alpha level of 0.05 was used to define statistical significance. Multiple secondary analyses of individual items on the ADAS-

Cog and Cornell scales were performed for heuristic purposes, recognizing that the possibility of type I errors is enhanced when multiple comparisons are performed.

RESULTS

There were no statistically significant differences among the three treatment groups with regard to age and other demographic variables or with regard to pretreatment baseline scores on the ADAS-Cog and other clinical measures (Table 1).

Tolerability and Safety

Besipirdine was generally well tolerated. Of 184 patients assigned to besipirdine (5 mg b.i.d. or 20 mg b.i.d.), 163 (89%) completed Segment I treatment. Among 91 placebo patients, 86 (95%) completed Segment I. During Segment I, treatment-emergent signs and symptoms considered to be related to study drug were seen in 24% of placebo-treated patients, in 29% of those receiving 5 mg b.i.d. besipirdine, and in 34% of those receiving 20 mg b.i.d. besipirdine. The majority of the effects were nervous system signs and symptoms: 11% of the placebo group, 21% of the group receiving besipirdine at a dose of 5 mg b.i.d., and 23% of those on 20 mg b.i.d besipirdine. The most prevalent effects were agitation, depression, dizziness, insomnia, nervousness, and abnormal thinking. Study-drug-related postural hypotension occurred in 3% of patients on 20 mg b.i.d., 1% on 5 mg b.i.d., and none on placebo, but it did not result in discontinuation for any patient. There were no appreciable differences among the placebo and besipirdine treatment groups with regard to the incidence of abnormalities on laboratory tests.

TABLE 1. *Characteristics of patients at baseline by treatment group*

Characteristics	Placebo treatment group (n = 91)	Besipirdine treatment groups	
		5 mg b.i.d. (n = 90)	20 mg b.i.d. (n = 94)
Age (years)			
Mean	71.7	72.2	70.8
Range	49–89	54–91	49–89
Sex (%)			
Male	49	48	38
Female	51	52	62
Race (%)			
White	95	94	97
Black	4	2	3
Other	1	3	0
ADAS-Cog			
Mean	24.2	26.8	25.7
Standard error	1.18	1.33	1.35
Mini-Mental State Exam			
Mean	18.5	18.8	18.9
Standard error	0.48	0.50	0.52
Global Deterioration Scale			
Mean	4.4	4.4	4.4
Standard error	0.08	0.08	0.08
Modified Hachinski Ischemia Scale			
Mean	0.5	0.5	0.4
Standard error	0.08	0.08	0.08
Cornell Scale for Depression in Dementia			
Mean	2.4	2.1	2.4
Standard error	0.24	0.18	0.21

ADAS-Cog: Primary Hypothesis

There were 268 analyzable patients for whom ADAS-Cog data were available at baseline and who had at least one assessment during Segment I treatment (Table 2). Progressive deterioration over the 12 weeks of Segment I was observed in placebo-treated patients, whereas besipirdine-treated patients were essentially unchanged from baseline over the same period. In the intent-to-treat test of the primary study hypothesis, the difference in mean change scores between the placebo and the combined besipirdine treatment groups was 1.26 points on the ADAS-Cog scale, and it approached, but did not reach statistical significance ($p = 0.067$). When the same hypothesis was tested in analysis of patients who completed all ADAS-Cog assessments in Segment I ($n = 238$), the magnitude of the treatment difference at end point was 1.41 points, which was statistically significant ($p = 0.031$).

ADAS-Cog: Secondary Hypotheses

A secondary hypothesis was that efficacy apparent during Segment I would persist after discontinuation of treatment during Segment II. Among patients treated with besipirdine in Segment I and placebo in Segment II, the mean change from baseline at the Segment II end point was 0.53 ADAS-Cog points less than the mean change for patients treated with placebo in both Segments I and II, using the intent-to-treat analysis ($p = 0.55$). This difference indicates that less than half of the efficacy (1.26 points) apparent after 12 weeks of treatment persisted after a further 12 weeks without treatment. This negative result must be interpreted cautiously, however, because the number of analyzable patients in Segment II ($n = 229$) provides <80% statistical power to detect a treatment effect. The result was similar to that for Segment II completers ($n = 199$).

Secondary analyses made separate comparisons of the treatment groups taking besipirdine at the 5 mg and 20 mg b.i.d. doses with the placebo group. In the Segment I intent-to-treat analyses (Table 2), the magnitude of the mean change from baseline was essentially the

TABLE 2. *Segment I intent-to-treat analysis*

Outcome measure ± or value indicating deterioration)	Change from baseline to end point			
	Placebo treatment group	Besipirdine treatment groups		
		Combined	5 mg b.i.d.	20 mg b.i.d.
ADAS-Cog (+)				
Mean	1.33[a]	0.07	0.06	0.08
Standard error	(0.52)	(0.40)	(0.56)	(0.56)
n	90	178	86	92
ADAS-Noncog (+)				
Mean	0.02	0.39	0.55	0.24
Standard error	(0.28)	(0.28)	(0.40)	(0.39)
n	90	179	87	92
CIBIC-Plus (>4)				
Mean	3.9	4.1	4.1	4.1
Standard error	(0.08)	(0.06)	(0.09)	(0.08)
n	90	180	88	92
Cornell Scale for Depression in Dementia (+)				
Mean	–0.29	0.46[a]	0.60[a]	0.32
Standard error	(0.16)	(0.17)	(0.23)	(0.25)
n	90	178	87	91

[a]$p < 0.05$ for within-group paired t test, comparing baseline to end point. Comparisons between the besipirdine and placebo groups are discussed in the text (see Results).

same for both the 5 mg and 20 mg b.i.d. dosing regimens, and it did not differ significantly from the change for the placebo group. In the analyses of Segment I completers, displayed in Fig. 2, the difference after 12 weeks of treatment compared with placebo was 1.23 ADAS-Cog points for the 5 mg b.i.d. ($p = 0.097$) group and 1.58 points for the 20 mg b.i.d. group ($p = 0.036$). After an initial small improvement attributable to practice effect, the mean change in scores of the besipirdine and placebo treatment groups diverged over time through 12 weeks of treatment. Figure 3 displays the Segment II results for patients treated with besipirdine, 20 mg b.i.d., during Segment I who completed Segment II on placebo compared with patients who completed both Segments I and II on placebo, illustrating the convergence over time of the mean change in scores for the two groups.

Individual items on the ADAS-Cog scale for which the greatest differences were observed that favored the combined besipirdine treatment groups over the placebo group in the Segment I intent-to-treat analyses were word recognition memory (0.6 points, $p = 0.042$), word recall memory (0.3 points, $p = 0.057$), and comprehension of spoken language (0.2 points, $p = 0.048$). Significance of the statistical tests for individual items is

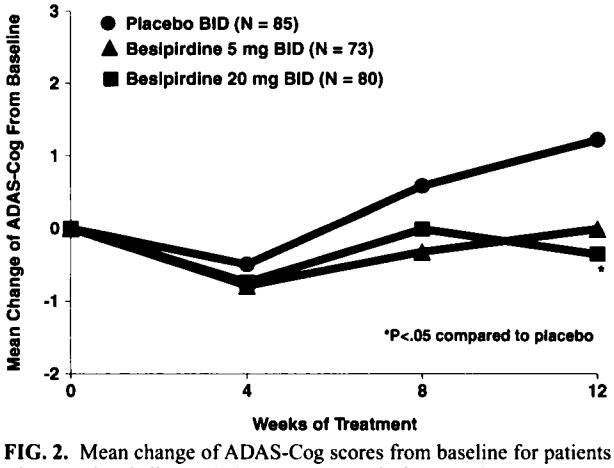

FIG. 2. Mean change of ADAS-Cog scores from baseline for patients who completed all ADAS-Cog assessments in Segment I.

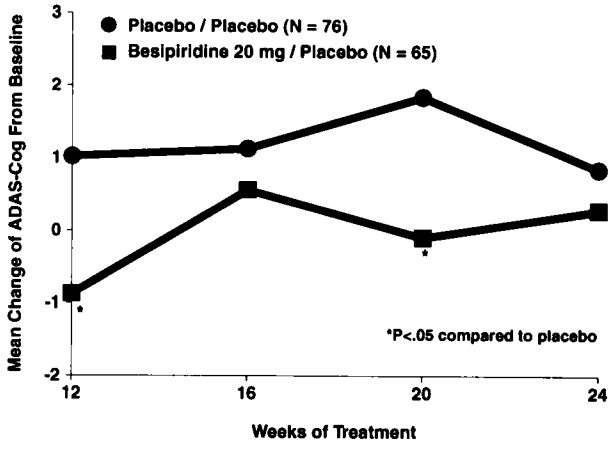

FIG. 3. Mean change of ADAS-Cog scores from baseline for patients who completed all ADAS-Cog assessments in Segment II. Placebo/Placebo represents patients who received placebo b.i.d. during both Segment I and Segment II. Besipirdine 20 mg/Placebo represents patients who received besipirdine, 20 mg b.i.d., during Segment I and placebo b.i.d. during Segment II.

uncertain because multiple comparisons enhance the possibility of type I errors. No difference for any item favored placebo by >0.1 point, and none approached statistical significance.

In the intent-to-treat analysis, 35% of patients treated with 5 mg b.i.d. besipirdine and 34% of patients treated with 20 mg b.i.d. besipirdine showed at least a two-point improvement compared with 26% of placebo-treated patients. Twenty percent of patients treated with 5 mg b.i.d. besipirdine and 23% of patients receiving 20 mg b.i.d. besipirdine improved by at least four points, compared with 14% of placebo-treated patients. None of these differences between the besipirdine and placebo groups were different by chi-square tests. The results did not differ appreciably when analysis was limited to Segment I completers.

CIBIC-Plus

A rating of 4 on the CIBIC-Plus scale reflects no change from baseline, a rating of 1 reflects marked improvement, and a rating of 7 indicates that the patient is markedly worse. More than 50% of patients were rated as unchanged at weeks 4, 8, and 12 of treatment. Mean ratings by treatment group across all Segment I and II assessments spanned the narrow range of 3.9–4.1, indicating that no substantial differences among treatment conditions were discerned by the clinician raters over this observation period. In the intent-to-treat analysis for Segment I, small (0.2 point) but statistically significant differences in favor of placebo were observed in the comparison with the combined groups ($p = 0.033$) and the 5 mg b.i.d. group ($p = 0.031$); a similar difference for the 20 mg b.i.d. group was not significant ($p = 0.108$). Similar comparisons for the Segment I completers indicated smaller treatment differences, none of which were statistically significant.

Secondary Outcome Measures

ADAS-Noncog

Results of the intent-to-treat analysis for Segment I are displayed in Table 2 for the placebo, combined, 5 mg b.i.d., and 20 mg b.i.d. treatment groups. None of the active versus placebo treatment differences were statistically significant.

BDS

The intent-to-treat analyses of change from baseline to the end point of Segment II for the combined, 5 mg b.i.d., and 20 mg b.i.d. treatment groups compared with placebo found no treatment differences that were statistically significant for either the IMC or the Dementia Score of the BDS.

Cornell Scale for Depression in Dementia

Results for the placebo, combined, 5 mg b.i.d., and 20 mg b.i.d. treatment groups in the intent-to-treat analysis of Segment I are shown in Table 2. Significantly higher ratings were observed compared with placebo for the combined treatment groups ($p = 0.006$), the 5 mg b.i.d. group ($p = 0.002$), and the 20 mg b.i.d. treatment group ($p = 0.046$). Among the individual items on the Cornell scale, greater worsening from baseline was observed in the combined besipirdine treatment groups than in the placebo group in the Segment I intent-to-treat analysis for ratings of appetite loss ($p = 0.017$), loss of interest ($p = 0.024$), and lack of energy ($p = 0.040$). Other items with greater worsening in the besipirdine group of comparable magnitude, but without statistically significant differences from placebo, were early morning awakening, agitation, and irritability.

DISCUSSION

The results of this study suggest that besipirdine may have a modest effect on the cognitive deficit in patients with AD. Whereas patients treated with placebo deteriorated over 3 months (12 weeks), patients treated with besipirdine remained essentially unchanged. This result was not demonstrated definitively in the intent-to-treat analysis, but it was supported by a prospectively planned analysis of patients who completed all Segment I ADAS-Cog assessments. The results of the withdrawal of treatment in Segment II indicate that the efficacy apparent at the end point of Segment I did not persist after 12 weeks without treatment, consistent with a symptomatic rather than neuroprotective effect. The magnitude of cognitive benefit apparent after 12 weeks was small, and the possibility that treatment for longer periods or with higher doses may enhance the efficacy of besipirdine has not been supported by a subsequently completed 24-week study.

Changes observed on the ADAS-Cog scale were not detected on the CIBIC-Plus global rating. It is possible that the magnitude of the cognitive effect was not sufficiently large for the clinicians to discern it in their global assessment. Another factor potentially affecting the CIBIC-Plus ratings is that there apparently is a clinically small but statistically significant adverse effect on mood and behavior, which was evident in spontaneous reports and was measured by the Cornell scale over the 12 weeks of treatment with besipirdine. Because

the effect of besipirdine on norepinephrine uptake and release indicates a potential for modifying mood, to which the ADAS-Noncog may be insensitive, the Cornell scale was used as a secondary outcome measure in the study. Patients with clinically important depression were excluded from the study, and scores on the Cornell scale during the study reflected mood status well below the threshold for diagnosis of major depression. Nevertheless, treatment-emergent symptoms detected by the Cornell scale, and also by spontaneous adverse event reporting, may have offset the apparent cognitive improvement in determining the global ratings recorded through the CIBIC-Plus. These symptoms may be attributed to either the adrenergic effect (agitation, irritability, sleep disturbance) or the cholinergic effect (depression, appetite loss) of besipirdine.

A previous attempt at combined cholinergic and adrenergic treatment of AD was reported by Bierer et al. (1993). They performed a double-blind crossover study of the alpha-2 adrenergic antagonist yohimbine and the cholinesterase inhibitor physostigmine. Yohimbine was given in single doses of 10 mg or 20 mg, crossed with placebo. Concurrently, patients were administered 2 mg physostigmine every 2 hours or placebo every 2 h. In 10 patients with AD, no difference in effect on the ADAS was observed between placebo and yohimbine or the combined treatment. A small physostigmine effect was demonstrated in comparison with placebo. One patient had a 3-h episode of chest pain with concurrent p-wave inversion in lead V_2 during dosing with 10 mg yohimbine and physostigmine. A myocardial infarction was excluded by serial electrocardiograms and enzyme tests. Although this study failed to find a treatment effect using combined cholinergic and adrenergic drugs, the conclusion is limited because only single doses of yohimbine were studied. It is possible that treatment effects would emerge during chronic dosing, but such an investigation is contraindicated by the occurrence of chest pain after a single dose.

Suronacrine, a derivative of the cholinesterase inhibitor velnacrine (Shutske et al., 1989), inhibits presynaptic uptake of norepinephrine as well as inhibiting acetylcholinesterase. Suronacrine was studied in 16 patients with AD (Huff et al., 1991), 12 of whom received ascending doses of suronacrine over 6 days and then 100 mg b.i.d. for 4 days, and four of whom received placebo. Slight improvements were observed on the ADAS and global rating in patients receiving suronacrine, but they were not statistically distinguishable from results in the placebo group. One patient discontinued treatment owing to dizziness, epigastric pain, irregular pulse, and a mild increase in blood pressure. Other patients experienced mild orthostatic and gastrointestinal symptoms that did not require discontinuation of treatment. Further study with higher doses of suronacrine was not pursued because plasma levels varied substantially, suggesting that for many patients tolerability would be poor at higher doses.

Wesseling et al. (1984) reported less deterioration on a rating scale and on a memory test in 14 patients with AD treated with 4-aminopyridine, 10 mg b.i.d., compared with placebo for 12 weeks. Davidson et al. (1988) studied another 14 patients with AD who were given 4-aminopyridine in doses of 2.5 mg, 5 mg, and 10 mg b.i.d. for 4 days each. The optimum dose selected for each patient was studied against placebo for 4 days in a replication crossover test. No effect on the ADAS was detectable.

In comparison with several of these previous attempts at treatment of multiple neurotransmitter deficiencies in AD, besipirdine was better tolerated, permitting clinical use of doses predicted to have efficacy based on animal models. Besipirdine has more clearly demonstrated adrenergic activity than 4-aminopyridine, and it thus provides a better test of the multiple neurotransmitter treatment approach. The suggestion of small cognitive benefits, combined with adverse effects on mood and behavior, associated with besipirdine treatment does not support the conjecture that multiple neurotransmitter treatment strategies may be more efficacious than monotherapy.

A high ratio of adrenergic to cholinergic potency may have resulted in the adverse effects of besipirdine, and it remains possible that a different proportion of adrenergic and cholinergic effects may confer an improvement over cholinergic monotherapy. Because the sample size does not provide sufficient statistical power for analysis of subgroups characterized by age, genetic features, or other sources of heterogeneity among patients with AD, the present study also does not exclude the possibility that subpopulations of AD patients may respond to combined cholinergic and adrenergic treatment.

Although the present study failed to demonstrate persistence of efficacy after withdrawal of treatment, it provides a practical example of the use of treatment withdrawal in an effort to distinguish neuroprotective from symptomatic treatment effects. Larger sample sizes and longer durations of both treatment and withdrawal intervals may be necessary in order to draw conclusive inferences using such study designs. The 3-month intervals used in the present study, however, may be adequate for phase II proof-of-principle studies of new treatment candidates in the therapy of AD.

Acknowledgment: This study was supported by Hoechst-Roussel Pharmaceuticals Inc. The authors thank the staffs of the Besipirdine Study Group and HRPI Clinical Neuroscience for assistance in performing the study; M. Chen, Ph.D., for statistical analyses; and S. Jaslove, Ph.D., for comments regarding the manuscript. The Besipirdine Study Group comprised, in addition to the authors, the following coinvestigators and study-site coordinators: J. Ravanelli-Meyer, R.N., C.R.C., Medical College of Wisconsin, Milwaukee, WI; A. Pedraza, M.D., and S. Bley, L.P.N., North Broward Medical Center, Pompano Beach, FL; J. Tomlinson, M.D., and B. Gullatt, R.N., C.N.A., Alzheimer's Diagnostic Clinic, Wichita Falls, TX; W. Richardson, R.N., California Clinical Trials, Beverly Hills, CA; R. Holub, M.D., and K. Hines, Neurological Associates of Albany, Albany, NY; C. Hirsch, M.D., and K. Cohen, L.P.N., Suncoast Medical Clinic, St. Petersburg, FL; S. Hersch, M.D., Ph.D., and J. Harrison, R.N., Emory University, Atlanta, GA; M. Keys, M.D., and S. Nelson, R.N., University of Cincinnati, Cincinnati, OH; M. Rymer, M.D., and P. Laubinger, R.N., M.B.A., St. Luke's Hospital, Kansas City, MO; J. Little, M.D., and K. Key, R.N., C.R.C., Austin Diagnostic Clinical, Austin, TX; R. Strub, M.D., and C. Benoit, C.R.C., Ochsner Clinic, New Orleans, LA; M. Rosenthal, D.O., and A. Ferber, Behavioral Medicine Resources, San Diego, CA; G. Gottlieb, M.D., and L. Ciesielski, M.S.N., R.N., University of Pennsylvania, Philadelphia, PA; M. Lloyd, R.N., Ph.D., Medical College of Pennsylvania, Philadelphia, PA; and A. Coyne, Ph.D., and S. Egan, R.N., University of Medicine and Dentistry of New Jersey, Piscataway, NJ.

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