

## Effects of N-PEP-12 on memory among older adults

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N-PEP-12 is a derivative of cerebrolysin, a brain-derived neuropeptide compound that has been approved for the treatment of Alzheimer's disease (AD) in more than 30 countries. N-PEP-12 is much less potent than cerebrolysin but it can be administered orally whereas the parent compound must be administered through multiple intravenous infusions. This study was undertaken to determine whether N-PEP-12 is effective in improving memory and other cognitive abilities among healthy older adults who have experienced 'normal' age-related memory loss. Subjects were 54 males and females, aged 50 years and older, who presented both subjective and objective evidence of memory loss since early adulthood. The study was a fully randomized, double-blind comparison of N-PEP-12 and placebo. Cognitive assessments were performed at baseline and following 30 days of treatment. The primary outcome measure was the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog) Memory score, with the Syndrom Kurz Test (SKT) test, digit cancellation, digit span, verbal fluency and clinical ratings as secondary

outcomes. N-PEP-12 treated subjects performed better than placebo-treated subjects on the ADAS-cog Memory score, the SKT, clinical ratings and some, but not other tests. N-PEP-12 may be an effective treatment for memory loss in healthy older adults. *Int Clin Psychopharmacol* 20:97-100 © 2005 Lippincott Williams & Wilkins.

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### Introduction

N-PEP-12, which is derived from cerebrolysin, is a peptide preparation produced enzymatically from purified nerve cell proteins and has multiple neurochemical and neurophysiological effects, many of which mimic the effects of nerve growth factor. For example, cerebrolysin has been shown to protect cholinergic neurones in the basal forebrain after fimbria-fornix transection (Akai and Hiruma, 1992), to diminish the neurotoxic effects of glutamate (Eder *et al.*, 2001) and to have anti-apoptotic effects on cortical neurones (Hartbauer *et al.*, 2001). Beyond such neuroprotective effects, cerebrolysin has neurochemical effects associated with acute improvement in memory, including increased glucose transport across the blood-brain barrier (Boado *et al.*, 1999) and increased activity of choline acetyltransferase (Akai and Hiruma, 1992). Furthermore, the compound produces long-lasting enhancement of synaptic transmission in the hippocampus, which is similar, and may be related to, long-term potentiation (Baskys and Wojtowicz, 1994). In all, the neurochemical and neurophysiological effects of cerebrolysin have been described in many models, both *in vitro* and *in vivo*, in more than 100 scientific publications.

The behavioural effects of cerebrolysin have been documented in many studies in animals (Gschanes and

Windisch, 1998), as well as in humans with Alzheimer's disease (AD) and other neurological disorders (Panisset *et al.*, 2002; Ruether *et al.*, 2001). Indeed, on the basis of large, carefully controlled clinical trials, cerebrolysin has been approved for therapeutic use by regulatory authorities in more than 30 countries.

However, cerebrolysin is probably not an appropriate treatment for disorders that are less profound than AD because it can only be administered through multiple (often 20) intravenous infusions. N-PEP-12 was developed by EBEWE Pharma (Unterach, Austria) as a compound that, although far less potent than cerebrolysin, can be administered orally. Cerebrolysin is safe to be administered at doses more than 20-fold greater than therapeutic doses (Leuschner, 1980a,b) and it was believed a less therapeutically potent oral derivative would be free of significant side-effects. This appears to be the case and the compound appears to have both neuroprotective effects on cortical cells and effects on memory performance in 'normal' aged rats (Hutter-Paier *et al.*, 2004). Eighteen-month-old rats fed N-PEP-12 for 3 months performed significantly better on Morris maze escape latency than did those given placebo. Furthermore, the animals were sacrificed after the experiment and a significant increase in synaptic density and plasticity was noted in several areas of the hippocampus

that are critical in learning and memory (e.g. CA1). These morphological changes were positively and significantly correlated with improved memory performance.

Preliminary studies of N-PEP-12 in humans have not been placebo-controlled but do demonstrate brain electrophysiological effects, as well as some suggestion of a clinical effect (Alvarez, 2003). Taken together, these data were considered to support a controlled trial of N-PEP-12 in adults with 'normal' age-related memory loss.

## Methods

### Subjects

Study participants comprised 54 male or female volunteers, aged 50 years or older, who presented with subjective complaints of memory loss and objective evidence of memory loss as demonstrated by a total score above 4 on the Syndrom Kurz Test (SKT; Erzigkeit, 1989). All individuals were in good physical health. Subjects meeting DSM-IV (American Psychiatric Association, 1994) and/or NINCDS/ADRDA (McKhann *et al.*, 1984) criteria for dementia were excluded, as were those with severe concomitant medical illness that could impair cognition. Subjects were recruited from areas near Bergondo, A Coruna, Spain and were seen at the EuroEspes Biomedical Research Centre under the direction of Dr Anton Alvarez. The study was approved by an Institutional Review Board and informed consent was obtained from all patients before initiation of any study procedure.

### Outcome measures

The primary outcome measure, identified before the study, was Memory score on the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog; Mohs, 1994). This score was obtained by summing scores on the three ADAS-cog memory tests. These are Word Recall, Word Recognition and Delayed Word Recall.

Secondary outcome measures included total score on the SKT test, a nine-scale memory test yielding a total score that is widely used to establish the effects of investigational compounds on cognition. Secondary measures also included as scores on two attentional tests: (i) a digit cancellation test that has been added to the expanded ADAS-cog for use in clinical drug trials (Mohs *et al.*, 1997) and (ii) the digit span subtest (forward and backward) from the Wechsler Adult Intelligence Scales-Revised (WAIS-R; Wechsler, 1981). Finally, a verbal fluency test was also performed. These secondary tests were added to determine if any cognitive effects of N-PEP-12 extended beyond memory.

In addition to the objective tests, the Sandoz Clinical Assessment-Geriatric scale (Shader *et al.*, 1974) was used to assess whether clinically significant effects can be

detected by subjects and clinicians. This scale assesses 18 symptoms (e.g. Mental Alertness), each described in specific detail, and all are summed for a total score. There is also a global 'Overall Impression' item. Each of the 19 items is rated on a 7-point scale (0 = Not present, 7 = Severe) by a trained rater based on an interview with the subject.

### Study design

The study was designed as a fully randomized, double-blind, placebo-controlled, parallel-groups trial comparing a single 90 mg daily dose of N-PEP-12 with placebo. Subjects were randomized on a 2:1 allocation ratio, N-PEP-12 to placebo, and treated for 30 days. Cognitive and clinical evaluations were performed at baseline and at the termination of treatment. Adverse effects were assessed through reports of adverse events and vital signs.

### Statistical analysis

The efficacy model was a univariate analysis of covariance to compare the N-PEP-12 and placebo groups at post-treatment on the primary and secondary outcome measures. If no significant differences were found between the two treatment groups with respect to age (a powerful predictor of test performance), the single covariate would be baseline performance. If the groups differed with regard to age, then that factor too was added to the model as a covariate. The single main effect in the model was 'Treatment' (N-PEP-12 versus placebo). *t*-tests were conducted to compare the two treatment groups on each reported adverse effect.

## Results

Fifty-four subjects were randomized to treatment, 36 to N-PEP-12 and 18 to placebo, and all subjects completed treatment. As shown in Table 1, there were no significant differences between treatment groups on relevant demographic variables or baseline test performance. The mean age in both treatment groups was slightly over 68 years and slightly more than two-thirds were female in each group. There were also no differences between study groups on height, weight or vital signs.

**Table 1** Baseline comparisons of subjects treated with N-PEP-12 and placebo

Variable	N-PEP-12 (n=36)	Placebo (n=18)	Significance
Age	68.2±8.8	68.2±11	0.99
Female (%)	69.4	66.6	0.86
ADAS-cog Memory score	15.4±4.1	15.3±4.0	0.94
SKT total score	6.8±5.2	6.9±7.2	0.96
Digit span	7.9±1.5	7.9±1.6	0.95
Verbal fluency			
Categories	12.0±3.2	11.6±4.3	0.71
Letters	18.0±10.2	16.2±8.3	0.52

Data are mean ± SD except where indicated.

Table 2 Comparison of N-PEP-12 and placebo on primary and secondary outcome measures

Primary outcome measure	N-PEP-12	Placebo	Significance	Favours
ADAS-cog Memory score	13.9 ± 0.33	15.5 ± 0.46	0.01	N-PEP-12
Secondary measures				
SKT total score	6.0 ± 0.44	8.2 ± 0.62	0.01	N-PEP-12
Digit cancellation errors	26.2 ± 1.21	26.4 ± 1.72	0.95	
Digit span	8.4 ± 0.20	7.8 ± 0.28	0.13	
Verbal fluency				
Categories	8.5 ± 0.47	8.7 ± 0.66	0.76	
Letters	18.8 ± 0.82	19.8 ± 1.16	0.49	
SCAG				
Overall Impression	2.4 ± 0.08	2.8 ± 0.11	0.01	N-PEP-12
Total Score	29.7 ± 0.41	31.6 ± 0.59	0.01	N-PEP-12

Data are mean ± SD except where indicated.

Table 3 Adverse event frequency by treatment group

Symptom/event	N-PEP-12 (n=36)	Placebo (n=18)	P-value
Agitation episode	1	0	
Anemia	1	0	
Dizziness	1	1	
Dyspepsia	2	0	
Face dysesthesia	1	0	
Headache	1	1	
Hypotension	0	1	
Loss of appetite	1	0	
Submandibular dysesthesia	0	1	
Total number (%)	8 (22%)	4 (22%)	0.99

Table 2 provides a comparison of the two treatment groups on the primary and secondary outcome measures. In all cases, means shown are adjusted for any baseline performance differences between groups.

A significant difference ( $P < 0.01$ ) favouring N-PEP-12 was found on the primary outcome measure, the ADAS-cog Memory score. On secondary measures, a significant difference ( $P < 0.01$ ) favouring N-PEP-12 was found on the SKT test. A trend favouring N-PEP-12 was seen on WAIS digit span but any differences were not significant. No differences were found on the verbal fluency measure or the digit cancellation test. Significant differences ( $P < 0.01$ ) favouring N-PEP-12 were seen on both the Total Score and Overall Impression on the SCAG, even though floor effects were seen on many symptoms unrelated to memory (e.g. Unsociability, Hostility, Uncooperativeness). Items on which highly significant differences were seen included 'Impairment of Recent Memory' and 'Impaired Mental Awareness.'

Adverse events reported in each treatment group are shown in Table 3. There were no significant differences between N-PEP-12 and placebo.

## Discussion

The results of the study suggest the N-PEP-12 may be a safe and promising compound for improving memory among older adults who have experienced 'normal,' age-related memory loss. It is of interest that subjects treated for 1 month with N-PEP-12 improved relative to subjects

treated with placebo both on objective and subjective assessments of memory. The vast majority of studies conducted in comparable populations have been negative and, in the sole clear exception (Crook *et al.*, 1991), objective changes were not accompanied by subjective improvement. Subjects performed better on neuropsychological tests but did not perceive any improvement, and nor did clinicians. It is also noteworthy that, in the current study, significant differences between treatment groups were seen, even though the sample size was relatively small and the treatment period was limited to 30 days.

The absence of a significant effect of N-PEP-12 on the digit cancellation, digit span and verbal fluency tasks suggests that the effects of the compound are specific to memory. Of course, there is a strong memory, as well as an attentional, component to digit span performance (Crook and Larrabee, 1988) and, on that measure, there was a trend favouring N-PEP-12. The digit cancellation test used in this study required that subjects bear in mind only two numbers for 60s as they searched for the numbers in an array. On that attentional task, there was no evidence of a treatment effect. There was also no evidence of a treatment effect on verbal fluency, which has long been accepted as a measure of frontal lobe function (Lezak, 1983).

As to the magnitude of the cognitive effects seen in this study, the difference between N-PEP-12 and placebo-treated subjects on the primary outcome measure, the ADAS-cog Memory Score, was 1.6 points. The ADAS-cog Memory score comprises 34 of the 70 possible points on the full ADAS-cog and effect sizes of 2–3 points on the full ADAS-cog have long been the basis for approval by regulatory authorities of the cholinesterase inhibitors now used to treat Alzheimer's Disease (Wolfson *et al.*, 2002). This effect size is generally observed only after extended treatment (usually 6 months) of hundreds of patients and is the result of deterioration in the placebo group rather than improvement in the group treated with active drug (Wolfson *et al.*, 2002). Thus, an effect size of 1.6 on the ADAS-cog Memory Score alone, after 1 month of

treatment in a relatively small sample, compares very favourably with the effect size of drugs currently approved to treat AD. In addition to comparisons with approved cognitive drugs, there are sizeable absolute increases in performance (e.g. a 27% increase in performance over placebo on the SKT).

Many questions regarding N-PEP-12 remain unanswered. Was the dose and dosage regimen chosen for this study optimal? Is 30 days treatment required to detect a drug effect or does N-PEP-12 act more quickly? Would more profound effects be observed with longer dosing? Would the magnitude of an effect be greater when measured with advanced computerized test procedures? Could memory training procedures administered simultaneously produce additive or even synergistic effects? Would effects be seen in other populations? Innovative studies aiming to answer such questions are now being planned.

In summary, many questions about N-PEP-12 remain unanswered, but the findings of the present study are encouraging.

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