DRUGS EXPTL. CLIN. RES. **XXX**(1) 27-33 (2004)

TREATMENT OF ALZHEIMER'S DISEASE WITH STABILIZED ORAL NICOTINAMIDE ADENINE DINUCLEOTIDE: A RANDOMIZED, DOUBLE-BLIND STUDY

DEMARIN V.,[1] PODOBNIK SARKANJI S.,[1] STORGA-TOMIC D.,[2] KAY G.[3]

1) Department of Neurology, Sestre Milosrdnice University Hospital, Zagreb, Croatia.
2) Institute for Medical Chemistry, University of Graz, Graz, Austria.
3) Department of Neurology, Georgetown University School of Medicine, Washington, D.C., USA.

Summary: *This study was designed to evaluate the effect of stabilized oral reduced nicotinamide adenine dinucleotide (NADH) on cognitive functioning in patients with Alzheimer's disease (AD). NADH is a coenzyme that plays a key role in cellular energy production and stimulates dopamine production. In previous trials NADH has been shown to improve cognitive functioning in patients with Parkinson's disease, depression and AD. The present trial was a randomized, placebo-controlled, matched-pairs, double-blind, 6-month clinical study. Patients with probable AD (n = 26) were randomized to receive either stabilized oral NADH (10 mg/day) or placebo. Twelve pairs of subjects were matched for age and baseline total score on the Mattis Dementia Rating Scale (MDRS) and the Mini Mental State Examination. After 6 months of treatment, subjects treated with NADH showed no evidence of progressive cognitive deterioration and had significantly higher total scores on the MDRS compared with subjects treated with placebo ($p < 0.05$). Analysis of MDRS subscales revealed significantly better performance by NADH subjects on measures of verbal fluency ($p = 0.019$), visual-constructional ability ($p = 0.038$) and a trend ($p = 0.08$) to better performance on a measure of abstract verbal reasoning. There were no differences between groups in measures of attention, memory, or in clinician ratings of dementia severity (Clinical Dementia Rating). Consistent with earlier studies, the present findings support NADH as a treatment for AD.*

Introduction

Reduced nicotinamide adenine dinucleotide (NADH) is a coenzyme that plays a key role in cellular energy production by oxidative phosphorylation (1). Furthermore, NADH stimulates dopamine production (2) and regenerates tetrahydrobiopterin (3), an essential cofactor of tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis, and prevents nitration of this enzyme by peroxynitrite (4). In open label clinical trials, NADH has been shown to improve cognitive functioning in patients with Parkinson's disease (5), depression (6) and Alzheimer's dis-

Address for correspondence: V. Demarin, Sestre Milosrdnice University Hospital, 10 000 Zagreb, Croatia.
Tel/Fax: +38513768282
E-mail: vida.demarin@zg.tel.hr

0378-6501/2004/1 00027+6 $02.50/0

ease (AD) (7). The ability of stabilized oral NADH to be absorbed and to cross the blood brain barrier was recently demonstrated by Rex *et al.* (8), who showed a 20% increase in the NADH level in rat cortex following oral administration of NADH. Previously, following 1-week intravenous infusion of NADH to Parkinson's disease patients, Kuhn *et al.* (9) reported increased levels and bioavailability of plasma levodopa (AUC 25% increase and C_{max} 40% increase). Although cholinergic dysfunction, a primary manifestation of AD, is the main target of current pharmacologic treatment of AD, there is considerable evidence of dysfunction of the dopaminergic neurotransmitter system in AD (10). As the dopaminergic system is important not only for motor performance but also for cognitive functions, attempts to restore deficits of the dopaminergic system are and should be further considered as a therapeutic approach for AD (11, 12).

Based on these findings, NADH may be a suitable candidate for further investigation in the treatment of AD based on its ability to facilitate dopamine synthesis.

As a prerequisite for a study with human subjects, Birkmayer and Nadlinger (13) investigated the safety and potential toxicity of the stabilized, orally absorbable form of reduced NADH in rats in order to demonstrate the safety of this drug in a chronic study. There were no deaths associated with the study drug and no treatment-related clinical signs. No differences were observed between the control and the treated groups in terms of hematology or clinical chemistry parameters. There was no apparent treatment-related effect on urine analysis parameters or on either absolute or relative organ weight. Furthermore, no macroscopic evidence of specific target organ toxicity associated with the test drug was observed. The results of this study and the fact that the daily dose of 5 mg per day administered to rats in this study corresponds to a dose of 175 mg per day in a 70-kg human indicate that stabilized oral NADH 5 mg tablets can be generally regarded as safe.

A series of studies have examined the use of NADH in AD. The first study was an open-label 8- to 12-week trial with oral stabilized NADH (ENADA®, Prof. Birkmayer Gesundheitsprodukte GmbH, A-1090 Wien, Schwarzspanierstr. 15, Austria; 10 mg per day) in 17 patients with AD. The results showed improvement (8.35X points ± 2.45; $p < 0.001$) on the Mini Mental State Examination (MMSE) and on the Global Deterioration Scale (1.82 points ± 0.39, $p < 0.001$) (7).

The initial double-blind, placebo-controlled, parallel group study was a pilot project designed to evaluate the effect of NADH on cognitive functions particularly sensitive to changes in AD severity (*i.e.*, verbal fluency and verbal memory). Primary outcome measures were the Hopkins Verbal Learning Test (14) and Verbal Fluency Test (15). Seventeen subjects (NADH, $n = 9$; placebo, $n = 8$) completed the study. Randomization groups differed at baseline on measures of overall verbal memory, dementia rating and attention. After 6 months of treatment, NADH subjects improved and placebo subjects declined on measures of verbal memory ($p < 0.04$). Similarly, improved verbal fluency was found for NADH subjects and decreased fluency was found for placebo subjects at 6 months ($p < 0.04$). The baseline differences in cognitive functioning indicated the need for better subject matching in subsequent small population trials (16).

The next study was conducted with 22 AD patients (NADH, $n = 12$; placebo, $n = 10$) with the same cognitive outcome measures. Groups were matched for baseline on the MMSE and age. The results demonstrated a trend for better verbal fluency ($p = 0.056$) and for better overall performance on the Mattis Dementia Rating Scale (MDRS) (particularly the fluency and memory measures). However, the results showed significant baseline differences between placebo subjects and NADH subjects on the MDRS, confounding interpretation of the results (16).

The present study was designed to rigorously match subjects *a priori* on the MDRS allowing for better analysis of the impact of NADH on cognitive functioning in AD.

Patients and methods

Patients were recruited from the outpatient clinic of the Neurology Department, University Hospital, Zagreb, Croatia and were enrolled in the study if they met the following criteria: diagnosis of probable AD according to criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association, age between 50 and 80 years, MMSE score of between 13 and 25, MDRS between 70 and 125, naive to treatment with NADH and no use of donepezil or tacrine within 6 months of enrollment in the study. Patients were not required to discontinue other medications. Seventy-five patients were screened and 25 met all the inclusion criteria. Informed consent was provided by caregivers and by patients capable of providing consent before entering into the study. The study was approved by the Croatian Ministry of Health.

In this double-blind, placebo-controlled, matched-pairs study, each patient made eight visits to the clinic over 6 months to examine the effects of stabilized oral NADH on cognitive functioning and to monitor for adverse effects and compliance. The primary outcome variable, chosen *a priori,* was the MDRS. After a 2-week drug compliance trial (passed by all patients), participants underwent a neurological examination, routine blood and urine laboratory tests and baseline cognitive testing. Random allocation to the two groups was computer-generated offsite; the key was stored at Birkmayer Institute (Vienna, Austria) until the end of the study. Patients were randomly assigned to receive NADH 5 mg (ENADA®, Prof. Birkmayer Gesundheitsprodukte GmbH, Wien, Austria), 2 tablets QD (n = 13) or matching placebo tablets (n = 13). Patients were monitored by neurological examinations at the eight study visits, and both patients and caregivers were questioned about adverse effects.

Neuropsychological testing was repeated at 10 weeks and 6 months, using the following measures: Hopkins Verbal Learning Test (14), Verbal Fluency Test (15), CogScreen® Matching to Sample Test (17) and Clinical Dementia Rating Scale (18).

MDRS (19) testing was performed at baseline and after 6 months. The primary outcome measure was total score on the MDRS.

We hypothesized that NADH would reduce deterioration and possibly improve cognitive functioning in AD. The analytic approach employed to test this hypothesis was Student's t-test for paired samples (SPSS-PC Version 10.1) comparing mean change from baseline to 6 months.

Results

Twenty-six patients were matched *a priori* into 13 study pairs. One patient was withdrawn from the study because of psychiatric hospitalization prior to initiating treatment. The matched subject of this pair was allowed to participate in the study but data for this subject is not included in the analyses (Fig. 1). All analyses were conducted with the 12 remaining pairs. The age range of the 24 patients included in the analyses was from 52 to 79 years; the median age was 68.5 years. The MMSE scores at baseline ranged from 13 to 25 with a median of 19.2. The MDRS scores at baseline ranged from 74 to 125 with a median of 107. The randomized groups were not statistically different with respect to age, gender, months since diagnosis, or dementia scores on the MDRS and MMSE at baseline.

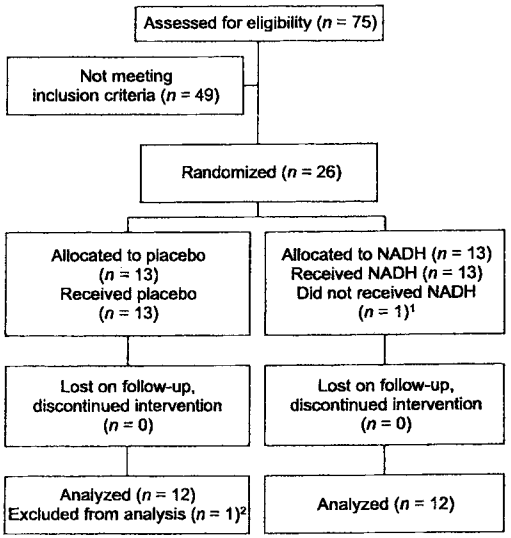

Fig. 1 Study participant flow. [1]Psychiatric hospitalization prior to treatment initiation. [2]No matched reduced nicotinamide adenine dinucleotide (NADH) subject.

Table I shows the findings for the two groups at baseline and 6 months. Change from baseline scores (6 months from baseline) revealed a mean increase in MDRS total score for NADH subjects of 1.25 (± 1.94) and a mean decrease in MDRS total score for placebo subjects of -7.92 (± 5.05) (Fig. 2). The difference between NADH and placebo groups in the primary outcome measure was significant ($T = 1.85$, $df = 11$, $p < 0.05$). Analysis of MDRS subscales revealed that the group differences in the MDRS total score were primarily due to the initiation/preservation scale ($T = 2.35$, $df = 11$; $p = 0.019$), the construction scale ($T = 1.97$, $df = 11$, $p = 0.038$) and the conceptualization scale ($T = 1.51$, $df = 11$; $p =$

Table I *Changes in the Mattis Dementia Rating Scale (total and subscales) after 6 months of treatment with 10 mg oral reduced nicotinamide adenine dinucleotide (NADH)*

	Changes in score		
	p-value	Group 1 stabilized oral NADH	Group 2 Control
MDRTOT28	0.046	-1.25	7.92
MDRIP28	0.019	-0.17	3.17
MDRCCP28	0.80	-0.67	2.25
MDRCON28	0.038	-0.08	0.75

MDRTOT28 = Mattis Dementia Rating Scale (MDRS) total score; MDRIP28 = MDRS Initiation/Perseveration subscale; MDRCCP28 = MDRS conceptualization subscale; MDRCON28 = MDRS construction subscale.

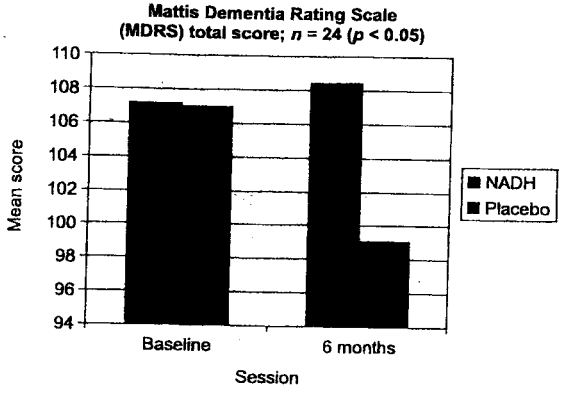

Fig. 2 Mattis Dementia Rating Scale before and after 6 months' treatment with reduced nicotinamide adenine dinucleotide (NADH).

0.080) (Table I). The results show an 8-point deterioration for the placebo group and a 1.25 improvement for stabilized oral NADH at 6 months (relative to baseline) on the total score for the MDRS. The improvement was most evident in measures of verbal fluency (naming items that could be found in supermarkets) and on a measure of visual construction skill (*i.e.*, reproducing geometric designs). The improvement in the conceptualization scale approached significance. Importantly, improvement was observed not only in the mean effect of NADH but also in each member of the pairs. In 8 out of 12 pairs matched on the MDRS there was positive effect in favor of NADH ($p < 0.05$) (Fig. 3).

There was a significant difference between the pairs at baseline in the Clinical Dementia Rating Scale. However, the groups did not differ in initial MDRS scores or in memory testing.

Analysis of the secondary outcome measures showed no significant difference between the treatment groups. However, placebo subjects demonstrated a trend for greater decline in verbal memory than NADH subjects. After 6 months of treatment stabilized oral NADH patients showed less deterioration in total recall on the Hopkins Verbal Learning Test (1 item fewer) *versus* placebo subjects (2.4 fewer items), slightly improved verbal recognition memory (0.33 items) *versus* deterioration in placebo subjects (1.25 fewer correct recognitions), less deterioration in verbal associative fluency (1.83 items) *versus* placebo subjects (2.75 items), less deterioration of categorical fluency (0.55 items) *versus* placebo subjects (1.09) items and a slight improvement (or no change) in the Clinical Dementia Rating Scale (0.04 points lower) *versus* placebo subjects (0.09 points higher, indicating very slightly deterioration). On the MDRS, nonsignificant findings were slight improvement on the memory scale for stabilized oral NADH (0.42 points, slight deterioration in placebo patients (0.08 points) and less deterioration on the attention scale (0.08 points lower) *versus* placebo subjects (1.67 points lower).

Medication compliance/adverse events. Overall medication compliance was 100%, based on pills returned and caregiver reporting. There were no adverse reactions related to the use of the study drug observed by caregivers or reported to examiners. Furthermore, the investigators observed no abnormalities in blood chemistries, complete blood counts, or electrocardiogram findings.

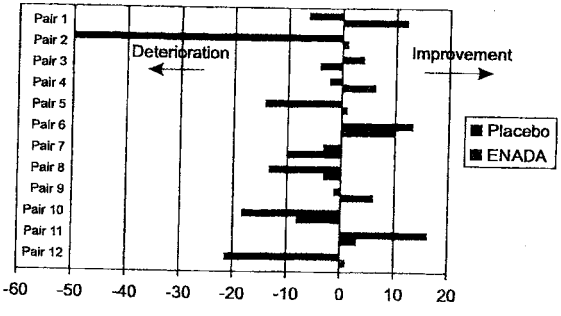

Fig. 3 Mattis Dementia Rating Scale 6-month change from baseline.

Discussion

The findings of this double-blind, placebo-controlled study are encouraging and consistent with those of earlier open label and double-blind studies in showing a beneficial effect of NADH on cognitive functioning in AD (7, 16).

From the previous studies we learned that careful matching of patients in cognitive variables prior to randomization is essential for valid comparison of the treatment groups.

On the measure of the validated and generally accepted measure of dementia, the MDRS, AD patients receiving stabilized orally absorbable NADH showed significantly better performance (compared with baseline) after 6 months of double-blind treatment than patients receiving placebo. The MDRS subscales that contributed to the total score difference were measures of verbal fluency, constructional ability and conceptual ability. At this point, it is difficult to directly compare the present findings with those reported in AD clinical trials using other therapies and outcome measures (20). However, no clinical studies using NADH have reported adverse or side effects during the 6-month treatment period (21).

In contrast to the placebo group, no statistically significant decline was observed on any of the cognitive testing batteries in patients receiving NADH. However, statistically significant improvements in certain cognitive functions were observed. These findings indicate that NADH could not only help to stabilize the disease but could also improve certain cognitive functions in AD patients. The results of this study confirm and extend previous findings on the beneficial effect of NADH on cognitive functioning in AD patients.

Acknowledgments

This study was supported by a grant from Birkmayer Institute for Parkinson's Therapy.

References

(1) Stryer L. *Oxidative phosphorylation.* In: "Biochemistry." Second Edition. W.J. Freeman and Co., San Francisco, 1981, pp. 307-329.

(2) Vrecko K., Storga D., Birkmayer J.G.D., et al. *NADH stimulates endogenous dopamine biosynthesis by enhancing the recycling of tetrahydrobiopterin in rat phaeochromocytoma cells.* Bioch. Biophys Acta., **1361**, 59, 1997.

(3) Vrecko K., Birkmayer J.G.D., Krainz J. *Stimulation of dopamine biosynthesis in cultured PC12 phaeochromocytoma cells by the coenzyme nicotinamide adeninedinucleotide (NADH).* J. Neural. Transm., **5**, 147, 1993.

(4) Kuhn D.M., Geddes T.J. *Reduced nicotinamide nucleotides prevent nitration of tyrosine hydroxilase by peroxynitrite.* Brain Res., **933**, 85, 2002.

(5) Birkmayer J.G., Vrecko C., Volc D., Birkmayer W. *Nicotinamide adenine dinucleotide (NADH) – a new therapeutic approach to Parkinson's disease. Comparison of oral and parenteral application.* Acta Neurol. Scand. Suppl., **146**, 32, 1993.

(6) Birkmayer W., Birkmayer J.G.D. *The coenzyme nicotinamide adenine dinucleotide (NADH) as biological antidepressive agent experience with 205 patients.* New Trends Clin. Neuropharmacol., **5**, 75, 1991.

(7) Birkmayer J.G. *Coenzyme nicotinamide adenine dinucleotide: New therapeutic approach for improving dementia of the Alzheimer type.* Ann. Clin. Lab. Sci., **26**, 1, 1996.

(8) Rex A., Hentschke M.P., Fink H. *Bioavailability of reduced nicotinamide-adenine-dinucleotide (NADH) in the CNS of the anaesthetized rat measured by laser-induced fluorescence spectroscopy.* Pharmacol. Toxicol., **90**(4), 220, 2002.

(9) Kuhn W., Müller T.H., Winkel R., et al. *Parenteral application of NADH in Parkinson's disease: Clinical improvement partially due to stimulation of endogenous levodopa biosynthesis.* J. Neural. Transm., **103**, 1187, 1996.

(10) Storga D., Vrecko K., Birkmayer J.G.D., Reibnegger G. *Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients.* Neurosci. Letts., **203**, 29, 1996.

(11) Gottfries C.G. *Pharmacology of mental aging and dementia disorders.* Clin. Neuropharmacol., **10**, 313, 1987.

(12) Piccinin G.L., Finali G., Piccirilli M. *Neuropsychological effects of L-deprenyl in Alzheimer's type dementia.* Clin. Neuropharmacol., **13**, 147, 1990.

(13) Birkmayer J.G.D., Nadlinger K. *Safety of stabilized, orally absorbable, reduced nicotinamide adenine dinucleotide (NADH): A 26-week oral tablet administration of ENADA®/NADH for chronic toxicity study in rats.* Drugs Exp. Clin. Res., **5**, 185, 2002.

(14) Brandt J. *The Hopkins verbal learning test: Development of a new verbal memory test with six equivalent forms*. Clin. Neuropsychologist, **5**, 125, 1991.

(15) Borkowski J., Benton A., Spreen O. *Word fluency and brain damage*. Neuropsychologia, **5**, 135, 1967.

(16) Kay G.G., Starbuck V.N., Demarin V., Podobnik S., Storga-Tomic D., Birkmayer J.G.D. *Cognitive improvement following treatment of Alzheimer's disease with stabilized oral NADH: Report of two six-month placebo-controlled, double-blind studies*. Presented at the Second Int. Conf. Signal Transduction. May 26-31, 2000, Dubrovnik.

(17) Kay G. "CogScreen." Aeromedical Edition. Professional manual, Odessa, FL, Psychological Assessment Resources, 1995, pp. 9-14.

(18) Berg L., Hughes C.P., Coben L.A., Danziger W.L., Martin R.L., Knesevich J. *Mild senile dementia of Alzheimer type: Research diagnostic criteria, recruitment, and description of a study population*. J. Neurol. Neurosurg. Psychiatry, **45**, 962, 1982.

(19) Lezak M.D. "Neuropsychological Assessment". Oxford University Press, New York, 1995, pp. 739-747.

(20) Mayeux R., Sano M. *Treatment of Alzheimer's disease*. N. Engl. J. Med., **341**, 1670, 1999.

(21) Forsyth L.M., Preuss H.G., MacDowell A.L., Chiazze L., Jr., Birkmayer G.D., Bellanti J.A. *Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome*. Ann. Allergy Asthma Immunol., **82**, 185, 1999.