

## ENADA/NADH improves cognitive impairment of Alzheimer patients

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Although significant progress has been achieved in revealing the etiology of AD, the search for efficient therapy stays the main goal. Nicotinamide adenine dinucleotide (NADH) is biologically identified as a cofactor necessary for a number of cellular actions, such as energy production, cell regulation and DNA repair, enhancement of cellular immune system and repair of oxidative damage. NADH is thought to be reduced in patients with neurodegenerative disorders and in clinical trials NADH has been shown to improve cognitive functioning in patients with AD, Parkinson's disease and in chronic fatigue syndrome.

This was randomized, double blind, placebo-controlled study with aim to establish whether NADH would improve cognitive functioning in patients with AD. 48 patients were randomized in the study, 24 were allocated to placebo and 24 to NADH treatment. Primary outcome measure was the difference in Mattis Dementia Rating Scale total score between baseline and after six months of treatment. The rest of cognitive tests included Hopkins Verbal Learning Test, Fuld Object Memory Test, Matching to Sample Test, Verbal Fluency Test, Clinical Dementia Rating Scale and Mini Mental Status Scale Exam.

NADH subjects improved significantly compared to placebo group in verbal fluency test. Placebo group experienced decrease of verbal fluency for 0.5 fewer words per minute and NADH group showed improvement of verbal fluency for 3.5 more words per minute ( $p=0.056$ ). Six months after baseline a mean increase in MDRS Total score for NADH group was 2.8 (+/- 1.76) and for placebo group a mean decrease in MDRS Total score -4.9 (+/- 3.13) points ( $p=0.022$ ). The results from this trial consistently demonstrate benefit of stabilized oral NADH on cognitive functioning in AD patients.

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## Introduction

Alzheimer's disease (AD) represents 45% of the total cumulative dementias (22-70% in individual studies). The estimated evidence is 123 new cases/100000 population/year. (1) Cholinergic system impairment in AD has been clearly established: acetylcholine and choline acetyltransferase are reduced in hippocampus and neocortex of patients with AD (2). Based on these findings, cholinesterase inhibitors were introduced for treatment of mild to moderate AD (2). In placebo controlled trials these drugs produced modest, but clinically significant difference compared with placebo, but adverse events limited their use (3,4,5,6). Other strategies for slowing decline in AD are based on trials that have shown possible benefit from use of neuroprotective agents such as selegiline and vitamin E (7). There is insufficient evidence to support the use of anti-inflammatory drugs and ginkgo biloba (8). Considerable evidence exists of dopaminergic neurotransmitter system dysfunction and oxidative stress in pathogenesis of AD (9,10,11). Previous SPECT studies clearly have shown the reduced activity in striatal regions in AD patients (12,13). Recent studies revealed also reduced activity of enzymes important for energy metabolism (NADH ubiquinone oxidoreductase, NADH diaphorase) in the brain of AD patients (12,13). Recent studies revealed also reduced activity of enzymes important for energy metabolism (NADH ubiquinone oxidoreductase, NADH diaphorase) in the brain of AD patients (14,15). As dopamine system is important not only for motor performance but also for cognitive functions, attempts to restore deficits of the dopamine system are considered as a therapeutic approach for AD (16,17). NADH (beta nicotinamide adenine dinucleotide reduced form, Co-enzyme 1) is present in all living cells and it plays a key role in cellular energy production by oxidative phosphorylation

(18). Furthermore, NADH stimulates dopamine production (19) and regenerates tetrahydrobiopterin (20), essential cofactor of tyrosine hydroxylase, the rate limiting enzyme in dopamine biosynthesis and prevents nitration of this enzyme (21). For treatment of neurodegenerative disorders the most interesting functions of NADH are antioxidant role, cell regulation and DNA repair. Stabilized oral NADH is able to cross the blood brain barrier (22). In open label clinical trials, NADH has been shown to improve cognitive functioning in patients with Parkinson's Disease (23), depression (24) and Alzheimer's Disease (AD) (25). Laboratory findings implicate the role of oxidative stress in pathogenesis of AD but still there is insufficient evidence to support the use of antioxidants because only few studies have been conducted and the results are inconclusive (8).

This study was conducted to determine whether the NADH, ENADA® may be of benefit in treating the symptoms of Alzheimer's disease and to determine safety when given to patients with Alzheimer's disease. Our hypotheses were: 1) NADH would arrest cognitive deterioration in AD and 2) NADH would improve cognitive functioning in AD.

## Methods

The study was designed as a six-month randomized, double blind, placebo controlled study. Patients with AD were recruited from the outpatient clinic of the Neurology Department, Sestre milosrdnice University Hospital, Zagreb, Croatia. Forty eight patients met inclusion criteria and were enrolled in the study. Patients with the diagnosis of probable AD, years of age between 50 and 80, Mini-mental status score between 10 and 25 and Clinical Dementia Rating Scale (CDR) between 1 and 2 were included. The diagnosis of AD was made according to the National Institute of Neurological and Cognitive disorders/ Alzheimer's Disease and

Related Disorders Association (NINCDS/ADRDA) criteria. All patients had CT scan of brain within the last 6 months demonstrating no tumor, communicating hydrocephalus, cerebral or subdural hemorrhage or focal stroke in either cerebral hemisphere. Patients with any clinically significant disease that could interfere with the study or with the interpretation of results were excluded. History of alcohol abuse or substance was exclusion criteria. All patients were naïve to treatment with NADH. Use of donepezil or tacrine was not allowed within the six weeks study, but patients were not required to discontinue other medications. Before entering into the study informed consent was obtained from legal representative or patient, if capable. The study was approved by the Ethical Committee of the Sestre milosrdnice University Hospital and by the Croatian Ministry of Health.

Every patient had physical and neurological examination, ECG, chest X-ray, blood and urine laboratory tests. Tests for cognitive evaluation included: Mattis Dementia Rating Scale (MDRS) (26), Hopkins Verbal Learning Test (27), Fuld Object Memory Test, Matching to Sample Test (28), Verbal Fluency Test (29), Clinical Dementia Rating Scale (30) and Mini Mental Status Scale (only on screening). For efficacy evaluation MDRS and Verbal Fluency Test were used as primary outcome measures. The study included a screening visit and eight testing sessions

at baseline, on week 2, 4, 6, 10, 14, 18 and after six months. Twenty-four patients were randomized to receive NADH 10 mg per day (ENADA® 5 mg, 2 tablets, Prof. Birkmayer Gesundheitsprodukte GmbH, A-1090 Vienna, Schwarzspanierstr. 15, Austria) and twenty-four to receive placebo. Safety evaluation included patient or caregiver report of any adverse event during the whole study, physical and neurological examinations and laboratory tests at screening, visit 1, 3, 4, 5, 6, 7, 8.

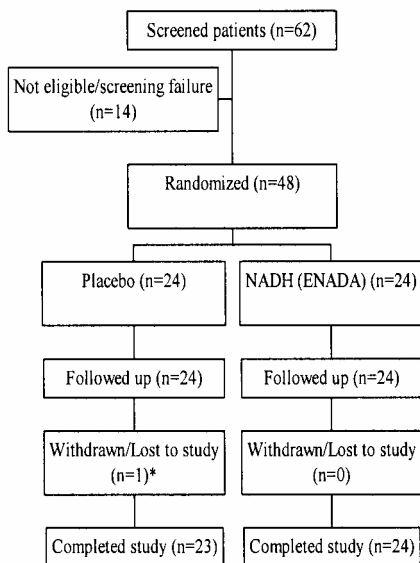
NADH and placebo group did not significantly differ with respect to age, gender and initial level of dementia measured by MMSE and MDRS.

The paired-samples T-test (SPSS-PC Ver. 10.1) was used in statistical analysis of mean change in primary outcome measures from baseline to 6 months.

## Results

Forty-eight patients were enrolled in the study, 29 males and 19 females. The age range was 54 to 80 years with median age of 69 years. The MMSE scores at screening ranged from 10 to 24 with a median of 18. The MDRS scores at baseline didn't significantly differ between the groups, mean score in placebo group was 95,6 and 96 in NADH group. 47 patients completed the study, one patient from the placebo group dropped due to traffic accident. Figure 1 describes the progression of patients through this trial.

Figure 1. Study Participant Flow (\*=patient withdrawn due to traffic accident)



### 6-Month Results

NADH subjects improved significantly compared to placebo group in verbal fluency test. While placebo group experienced decrease of fluency for 0.5 fewer words per minute. NADH group showed improvement of verbal fluency for 3.5 more words per minute ( $p=0.056$ ) (Figure 2).

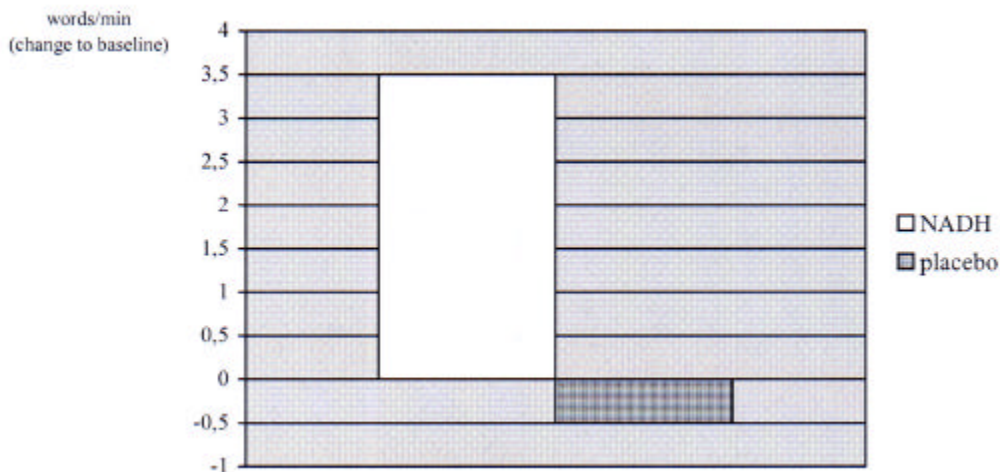


Figure 2: Verbal Fluency Test Change after 6 Months of Treatment

Results of the verbal recognition memory measure showed no significant advantage for NADH patients. Verbal memory of the Dementia Rating Scale showed advantage

for NADH patients, improvement for 1.2 points ( $p<0.04$ ) (Fig. 3). For NADH group six months after baseline a mean increase in MDRS Total score of  $2.8\pm 1.76$  was

found and for placebo group a mean decrease in MDRS Total score of  $-4.9 \pm 3.13$ . This difference in MDRS Total score six months after baseline was significant ( $p=0.022$ ) (Fig. 4). The subscales on the MDR that contributed to the total score difference were measures of verbal fluency, constructional ability and conceptual ability.

## Medication Compliance/Adverse Events

Very high medication compliance overall in the study (100%) was achieved, based on number of pills returned and caregiver reporting. During the whole study none of the patients experienced any adverse event related to the use of the study drug observed by caregivers or reported to examiners. Furthermore, no abnormalities in blood chemistry, hematology or ECG were observed by the investigators in any subjects.

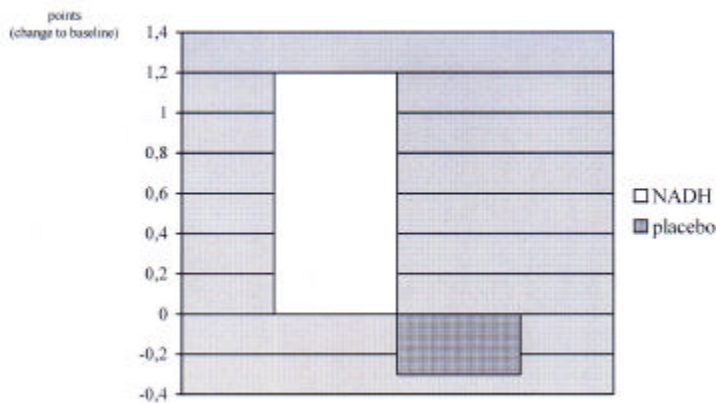


Fig. 3: Verbal Memory on the Dementia Rating Scale after 6 Months of Treatment

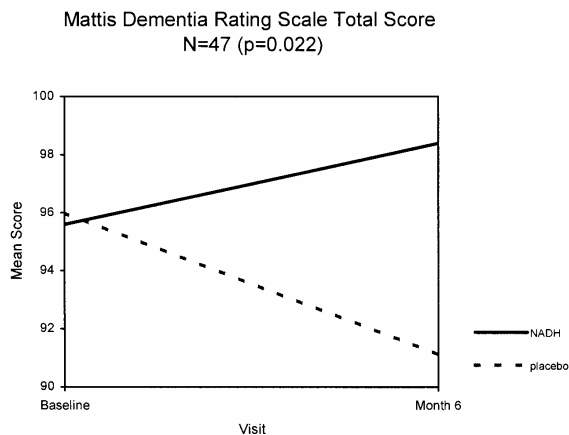


Figure 4: Mattis Dementia Rating Scale 6-month Change from Baseline

## Discussion

During six months of follow up, in patients receiving NADH no significant decline in cognitive functioning, measured by any cognitive tests had been observed. Placebo group experienced decline in cognitive functioning. AD patients receiving stabilized orally absorbable NADH showed significantly better performance on measures of verbal memory, verbal fluency and overall dementia rating following six months of double-blind treatment compared to AD patients receiving placebo. Results are encouraging and consistent with the results of the earlier studies with AD. In 1996, Birkmayer showed results of the pilot study, open label trial with NADH in the duration between 8 and 12 weeks. The minimal improvement on the Mini Mental State Examination was 6 points and maximum improvement 14 points with a mean value of 8,34 points (25).

Significantly better performance (compared to baseline) measured by MDRS following six months NADH treatment was result of double-blind study with stabilized orally absorbable NADH versus placebo (31). These findings are in concordance with hypothesis about role of oxidative stress, reduction of NADH diaphorase and oxidoreductase activity and impairment of dopaminergic system in the brain of the AD patients. Important to note, in all clinical studies using NADH no adverse or side effects have been observed during the treatment period of six months (32).

NADH is a natural substance present in all living cells so results about its safety are not surprising. In this as well as in previous studies patients or caregivers reported very good drug tolerance and no adverse events. The results from this trial consistently demonstrate benefit of stabilized oral NADH on cognitive functioning in AD patients, especially on verbal memory, verbal fluency and overall dementia rating. Since NADH is a natural product with proven effect and without any

known side effect, it should be strongly considered as mono- or additional therapy for treatment of Alzheimer's disease.

## Acknowledgements

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## References

1. AH Jorm, D Jolley. *Neurology* 51:728 (1998)
2. Victor M, Ropper AH. *Degenerative Diseases of the Nervous System*. Adams and Victor's principles of neurology. MJ Wonsiewicz, MP Medina, M. Nazarow. 7<sup>th</sup> edition. The McGraw-Hill Companies, Inc. USA 2001:1109.
3. Knapp M, Knopmann D, Solomon P, et al. for the Tacrine Study Group. *JAMA* 271:985 (1994).
4. Egger S, Lavy R, Sahakian B. *Lancet* 337:989 (1991).
5. Rogers S, Farlow M, Doody R, et al. and the Donepezil Study Group. *Neurology* 50:136 (1998).
6. Corey-Bloom J, Anand R, Veach J. for the ENA 713B352 Study Group. *Int J Geriatr Psychopharmacol* 1:55 (1998).
7. Sano M, Ernesto C, Thomas RG et al. *New Engl J Med* 336:1216 (1997).
8. Doody RS, Stevens JC, Beck RN et al. Dubinsky RM, Hays JA, Gwyther L, Mohs RC, Thal LJ, Whitehouse PJ, DeKosky ST, Cummings JL. *Neurology* 56:1154 (2001).
9. Halliwell B. *Drugs Aging* 18:685 (2001).
10. Engelhart MJ, Geerlings MI, Ruitenberg A, et al. *JAMA* 287 (24):3223 (2002).
11. Storga D, Vrecko K, Birkmayer JGD, Reibnegger G. *Neuroscience Letters* 203:29 (1996)
12. Pizzolato G, Chierichetti F, Fabbri M, et al. *Neurology* 47(4):1065 (1996).

13. Frisoni GB, Pizzolato G, Bianchetti A, et al. *Act. Neurol Scand* 89(3):199 (1994).
14. Kim SH, Vikolinsky R, Cairns A, et al. *Life Sci* 68(24):2741 (2001).
15. Jacobs RW, Farivar N, Butcher LL. *Neurosci Lett* 53(1):39 (1985).
16. Gottfries CG. *Clin. Neuropharmacol.* 10:313 (1987).
17. Piccinin GL, Finali G, Piccirilli. *Clin. Neuropharmacol* 13:147 (1990).
18. Stryer L. Oxidative Phosphorylation. In: WH Freeman and Comp ed. *Biochemistry*. 2<sup>nd</sup> ed. San Francisco: 1981:307-329.
19. Vrecko K, Storga D, Birkmayer JGD, et al. *Bioch. Biophys. Acta* 1361:59 (1997).
20. Vrecko K, Birkmayer JGD, Krainz J. J. *Neural. Transm. (P-D Sect)* 5:147 (1993).
21. Kuhn DM, Geddes TJ. *Brain Res* 933;1:85 (2002).
22. Rex A, Hentschke MP, Fink H. *Pharmacol Toxicol* 90(4):220 (2002).
23. Birkmayer JG, Vrecko C, Volc D, Birkmayer W. *Acta Neurol Scand Suppl* 146:32 (1993).
24. Birkmayer W, Birkmayer JGD. *Neuropharmacology* 5:75 (1991).
25. Birkmayer JG. *Ann Clin Lab Sci* 26:1 (1996).
26. Lezak MD. *Neuropsychological assessment*. New York: Oxford University Press, 1995.
27. Brandt J. *The Clinical Neuropsychologist* 5:125 (1991).
28. Kay G. *CogScreen – Aeromedical edition: professional manual*. Odessa, FL: Psychological Assessment Resources, 1995.
29. Borkowski J, Bento A, Spreen O. *Neuropsychologia* 5:135 (1967).
30. Berg L, Hughes CO, Coben LA, et al. *J Neurol Neurosurg Psychiatry* 45:962 (1982).
31. Mayeux R, Sano M. *N Engl Med* 341:1670 (1999).
32. Forsyth LM, Preuss HG, MacDowell AL, Chiazze L, Jr., Birkmayer GD, Bellanti JA. *Ann Allergy Asthma Immunol* 82 :185 (1999).

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