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Parenteral application of NADH in Parkinson's disease: clinical improvement partially due to stimulation of endogenous levodopa biosynthesis

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Summary. Exogenous application of levodopa is conventionally used to equalize the striatal dopamine deficit in idiopathic Parkinson's disease (PD). The stimulation of endogenous biosynthesis of levodopa via activation of tyrosine hydroxylase (TH) has been proposed as new therapeutic concept in PD. This may be achieved by exogenous supply with the reduced coenzyme nicotinamide adenine dinucleotide (NADH). Aim of this open prospective study was to investigate (1) the efficacy of a new developed, parenteral application form of NADH on Parkinsonian symptoms and (2) the influence of bioavailability of levodopa. 15 patients, suffering from idiopathic PD (11 male, 4 female, age: 61.40[mean] ± 10.27[SD] range: 44–74 years, Hoehn and Yahr stage: 3.03 ± 0.69, range 2–4) received intravenous infusions of NADH (10 mg a' 30 min) over a period of 7 days in addition to conventional Parkinsonian pharmacotherapy. Parkinsonian symptoms were scored before (day 1) and after NADH treatment (day 8). Levodopa plasma levels were estimated over a period of four hours on the day before and on the first day of NADH application by HPLC. Parkinsonian patients showed a significant response, evaluated by the Unified Parkinson's Disease Rating Scale Version 3.0 ($p = 0.025$; Wilcoxon test). Moreover application of NADH significantly increased bioavailability of plasma levodopa (AUC, $p = 0.035$; C_{max}, $p = 0.025$). In conclusion NADH in the used galenic form may be a potent stimulator of endogenous levodopa biosynthesis with clinical benefit for Parkinsonian patients.

Keywords: Parkinson's disease, NADH, endogenous biosynthesis of levodopa

Introduction

Presynaptic degeneration of dopaminergic nerve cells in the substantia nigra results in a striatal dopamine (D) deficit and loss of tyrosine hydroxylase (TH) in Parkinson's disease (PD) (Uitti and Calne, 1993). TH catalyzes conversion of the amino acid tyrosine into levodopa, the precursor of D. This metabolic reaction is the first and rate limiting step in the biosynthesis of D, which needs tetrahydrobiopterin (BH_4) as cofactor (Rausch et al., 1988). BH_4 is synthesized from dihydrobiopterin (BH_2) by the quinoid-dihydropteridine reductase, which is coupled on the NAD/NADH redox system (Iuvone et al., 1985). BH_4 is reduced in Parkinsonian brains, which could be due to decreased biosynthesis of BH_4 and/or a lack in the biological active form of BH_4 (Leeming et al., 1983; Nichol et al., 1985). In the second case application of NADH (reduced form) should induce an increased synthesis of BH_4 and subsequently via activation of TH an enhanced production of endogenous levodopa and D production in the brain. This hypothesis was confirmed in rat phaeochromocytoma cells (Birkmayer and Birkmayer, 1989). Moreover the clinical efficacy of intravenous and oral administration of NADH has been claimed in open prospective and retrospective trials on up to 884 Parkinsonian patients (Birkmayer et al., 1993, 1990, 1989). Aim of this open prospective study was to investigate (1) the efficacy of a new developed, parenteral application form of NADH on Parkinsonian symptoms and (2) the influence on bioavailability of levodopa.

Material and methods

Patients

15 patients (11 male, 4 female, age: 61.40[mean] ± 10.27 [SD] range: 44–74 years, mean duration of disease: 9.35 ± 7.39 range: 1–25 years, Hoehn and Yahr stage: 3.03 ± 0.69 range 2–4) without paroxymal on/off fluctuations and freezing phenomena were enrolled into the study, which was approved by the local ethic commitee.

Table 1. Study design. Levodopa levels at 6.30, 6.40, 6.50, 7.00, 7.10, 7.20, 7.30, 7.45, 8.00, 8.15, 8.30, 9.00, 9.30, 10.00, 10.30 a.m.; NADH infusion 6.30–7.00 a.m.; oral application of levodopa at 6.30 a.m.; conventional parkinsonian pharmacotherapy after 10.50 a.m.

Day	Levodopa plasma levels	Levodopa/benserazide (125 mg)	Conventional parkinsonian pharmacotherapy	UPDRS at 10.30	NADH
1	x	x	x	x	
2	x	x	x		x
3		x	x		x
4		x	x		x
5		x	x		x
6		x	x		x
7		x	x		x
8		x	x	x	x

Study design

The study was performed according to the time table, shown in Table 1. Levodopa plasma levels can easily disturbed by various factors (Gerlach et al., 1986). Therefore we measured levodopa plasma levels not on day 1 and 7, but on day 1 and 2 of the study, to minimize and eliminate possible disturbing circumstances, which may even occur under conditions of hospitalization of 2 and more days. Levodopa plasma levels were estimated at fixed time points from 6.30 a.m. to 10.30 a.m.. At 6.30 on each day (day 1 to 8) patients received one capsule, containing 100 mg levodopa and 25 mg benserazide (Madopar 125®). From day 2 to day 8 infusion of 10 mg NADH was applied between 6.30 a.m. and 7.00 a.m. without light exposure of NADH. After 10.50 a.m. patients received their conventional Parkinsonian pharmacotherapy. Earlier studies by Birkmayer et al. (1990, 1993) have shown, that clinical effects of NADH infusion treatment appear after a period of 7–14 days. Therefore patients' rating of clinical condition and degree of disability was determined at 10.30 on day 1 and day 8 using version 3.0 of the Unified Parkinson's Disease Rating Scale (UPDRS) (see Table 1).

Blood samples

10 ml venous blood samples for measurement of levodopa plasma levels were taken from an antecubital vein through an indwelling cannula kept patent by an infusion of heparin in saline solution (10 U/ml; Table 1). Blood (3 ml) was drawn with a separate syringe and discarded before each 10 ml specimen. Infusions of NADH were injected in the contralateral arm. Blood specimens were placed in EDTA-test tubes containing 100 µl of 0.5% sodium disulfite solution. The plasma obtained from rapid centrifugation was immediately frozen at −20°C until analysis. The content of levodopa was analyzed using reversed-phase HPLC and electrochemical detection (Gerlach et al., 1986). Concomittant pharmacotherapy, even regarding dosage and time of application remained stable throughout the whole study.

Application of NADH

NADH is highly instable in the presence of room temperature, humidity and light. Moreover the pH is very critical for the stability of NADH in solution. It is necessary to provide a range of pH 8–9 for sufficient stability of NADH in a suitable buffer (Mattern, 1995). Therefore lyophilized NADH (Boehringer, Mannheim) was applied into one of two chambers of a new developed syringe (Type "Injoject", Vetter Pharma, Ravensburg). The solvent in the second chamber of the syringe was a $NaCl/NaHCO_3$ buffer (0.030 mol/l). The lyophilized NADH was mixed with the solvent immediately before application providing an osmolarity of 290–320 mOsm in a pH-range of 8–9, which guarantees stability under isotonic conditions in comparison to plasma and other NADH-solutions, in which stability of NADH was not checked and no buffer as solvent was used to adjust the pH.

Statistics

The Wilcoxon sign rank test was used to compare the effects at the time points of measurement for the UPDRS-score (item 1–31). The baseline value day 1 at 10.30 a.m. was compared to the value of day 8 at 10.30 (Table 1). The Wilcoxon sign rank test was performed to compare $AUC_{6.30\,a.m.-10.30\,a.m.}$, C_{max} and t_{max} of levodopa plasma levels on day 1 and 2. Correlation analysis of the difference of UPDRS score on day 1 and day 8 with patients' age, mean duration of disease, Hoehn and Yahr stage, difference of $AUC_{6.30\,a.m.-10.30\,a.m.}$, C_{max} and t_{max} of levodopa plasma levels on day 1 and 2 was performed by Spearman rank correlation analysis.

Results

Parenteral application of 10 mg NADH over a period of 7 days significantly improved patients' UPDRS scores (Fig. 1, Table 2). No significant influence of patients' age (Spearman R = 0.445, p = 0.096), mean duration of disease (Spearman R = −0.011, p = 0.970) and Hoehn and Yahr stage (Spearman R = 0.054, p = 0.847) on the patients' response on NADH evaluated by the difference of the UPDRS score on day 1 and day 8 appeared.

Administration of NADH significantly increased levodopa bioavailability in plasma (Fig. 2: $AUC_{6.30-10.30\ a.m.}$ levodopa, Fig. 3: C_{max} levodopa, Table 2). No influence on t_{max} levodopa (p = 0.158) appeared by treatment with NADH.

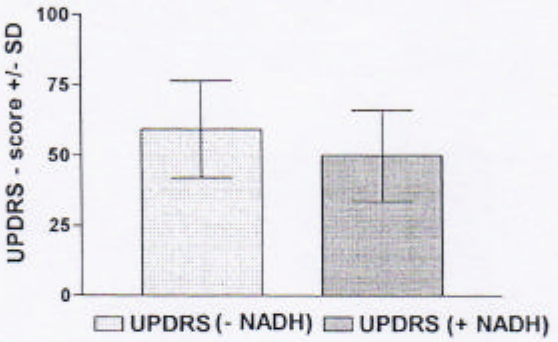

Fig. 1. Comparison of UPDRS score on day 1 at 10.30 a.m. with day 8 at 10.30 a.m. (day 1: mean: 59.3 ± 17.13 [mean] ± [SD], range: 33–87, day 8: mean: 50.03 ± 16.04, range: 29–79, difference: 9.26 ± 15.10, range: −13–50, p = 0.025)

Table 2. UPDRS, $AUC_{6.30-10.30\ a.m.}$ levodopa, C_{max} levodopa − data of patients. In patient 15 estimation of levodopa plasma levels was not performed

Patient	UPDRS	UPDRS (NADH)	$AUC_{6.30-10.30\ a.m.}$ levodopa	$AUC_{6.30-10.30\ a.m.}$ levodopa (NADH)	C_{max} levodopa	C_{max} levodopa NADH)
1	87	37	2029.029	2932.141	1431.124	1443.843
2	56	39.5	624.673	1858.512	412.376	1487.801
3	84	61	1564.67	976.9565	890.699	1204.097
4	65	51	1582.347	2247.367	1433.396	1215.28
5	79	67.5	1815.042	2223.876	793.96	1043.27
6	73	73	1400.719	2906.18	1186.898	2821.846
7	45	29.5	1494.437	2484.715	998.68	2263.704
8	63	51	3129.57	2948.469	1459.586	1453.12
9	49	43	1751.66	2131.032	556.303	1143.472
10	70.5	79	1917.295	1724.744	1590.994	1187.678
11	53.5	41.5	2552.775	2561.745	1017.913	1692.453
12	36	41.5	2152.753	3270.858	914.46	1486.609
13	39	35	3681.986	2942.235	2246.653	1844.339
14	33.5	32	1736.426	3245.109	508.979	1566.854
15	56	69				

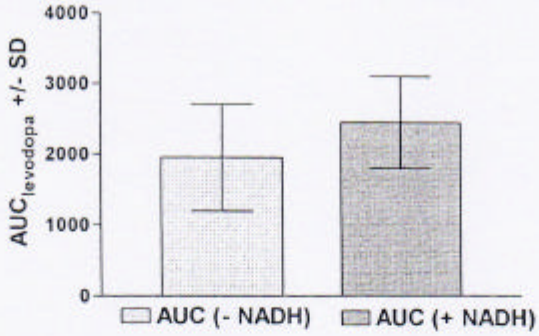

Fig. 2. Comparison of $AUC_{6.30\text{-}10.30\ a.m.}$ levodopa on day 1 with $AUC_{6.30\text{-}10.30\ a.m.}$ levodopa on day 2 during application of NADH (day 1: mean: 1959.5 ± 757.23 ng/l [mean] ± [SD], range: 624–3682 ng/l, day 2: mean: 2461.0 ± 649.79 ng/l, range: 976–3270 ng/l, difference: −501.47 ± 749.70, range: −1508–39 ng/l, p = 0.035)

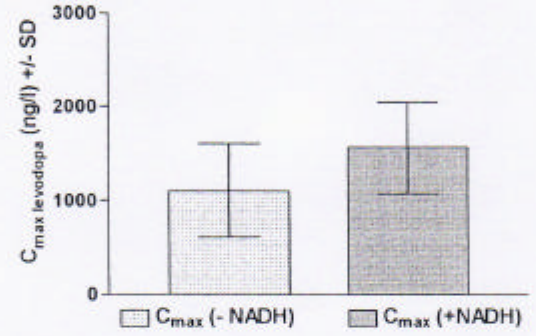

Fig. 3. Comparison of C_{max} levodopa on day 1 with C_{max} levodopa on day 2 during application of NADH (day 1: mean: 1103.0 ± 497.69 ng/l [mean] ± [SD], range: 412 − 2246 ng/l, day 2: mean: 1561.0 ± 484.06 ng/l, range: 1043–2821 ng/l, difference: −458.02 ± 638.80 ng/l, range: −1634–403 ng/l, p = 0.025)

No significant relation between difference of $AUC_{6.30\ a.m.-10.30\ a.m.}$ (Spearman R = −0.238, p = 0.383), C_{max} (Spearman R = −0.253, p = 0.413) and t_{max} (Spearman R = −0.067, p = 0.815) of levodopa plasma levels on day 1 and 2 and the patients' response on NADH evaluated by the difference of UPDRS score on day 1 and day 8 appeared.

Discussion

Our results of this pilot study with a new application form of NADH demonstrate that parkinsonian patients may respond to parenteral application of NADH. In contrast to the results of an open retrospective study on 885 Parkinsonian patients we found no significant influence of age and duration of disease on the therapeutic efficacy of NADH (Birkmayer et al., 1993). One double blind trial with a small group of patients (5 with NADH vs. 4 vs. placebo) showed also tendency to clinical improvement of Parkinsonian symptoms after intravenous infusion of 25 mg NADH (Dizdar et al., 1994).

This result was interpreted as clear placebo effect, because improvement of extrapyramidal symptoms was also found in the placebo group. In this study NADH was obtained by Sigma and dissolved in 100 ml of 0.9% sterile sodium chloride immediately before use (Dizdar et al., 1994). This pharmaceutical preparation of NADH is very sensitive to light exposure, room temperature and humidity (Mattern, 1995). In contrast to this study we used a new developed galenic form of NADH and protected NADH even during the infusion to light exposure (Mattern, 1995).

Regarding the significant increase of levodopa plasma levels after parenteral application of NADH, our study confirms results of in vitro trials with tissue cultures (Vrecko et al., 1993). The beneficial clinical effect resulting from the NADH application may be due to stimulation of endogenous biosynthesis of levodopa in the periphery (Birkmayer et al., 1990). But it cannot be excluded that the used NADH additionally stimulates the biosynthesis of levodopa in the nigrostriatal system, because this used preparation of NADH is able to penetrate the blood-brain barrier because of its lipophilicity. However it remains unclear to what extent (Mattern, 1995).

We conclude, that NADH in this galenic form may be a potent stimulator of endogenous levodopa biosynthesis with clinical benefit for Parkinsonian patients. One may speculate, that intravenous application of NADH may provide a sparing effect of exogenous levodopa supply in Parkinsonian patients. Further double-blind trials are neccessary to give more support for this new therapeutic concept of enzymatic stimulation of TH via dihydropteridine reductase.

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