Acta Neurol. Scand., 1989:126:183—187

Key words: Parkinson's disease, therapy, NADH, homovanillic acid, stimulation, DOPA biosynthesis

Stimulation of endogenous L-dopa biosynthesis — a new principle for the therapy of Parkinson's disease

The clinical effect of nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotidephosphate (NADPH)

G.J.D. Birkmayer, W. Birkmayer

Birkmayer Institute for Parkinson Therapy Vienna, Austria

ABSTRACT — The coenzyme nicotinamide adenine dinucleotide (NADH) has been used as a novel medication in 161 Parkinson patients in an open label trial. In all but 18 patients (11.2 %) an improvement in their disability was observed. 115 patients (71.4 %) showed a very good (better than 30 %) response, and 28 patients (17.4 %) a moderate response up to 30 %. The best results were obtained with a dose of 25 to 50 mg every second day by i.v. administration. Concomitantly with the improvement in disability, the urine HVA level increased significantly, indicating a stimulation of endogenous L-DOPA biosynthesis. 8 patients have been treated with nicotinamide adenine dinucleotidephosphate (NADPH), 5 of whom exhibited an improvement in their disability from 35 to 55 %. The other 3 showed a moderate response of 20 to 25 %. In all these patients an increase in the urine level of HVA was detected, reflecting elevated endogenous L-DOPA production.

Introduction

The biochemical cause of the motoric disability in parkinsonian patients is a shortage of the catecholamine dopamine in the basal ganglia of the brain. The immediate precursor of dopamine, L-DOPA, was the first rational therapy for Parkinson's disease, introduced by Birkmayer and his group in 1961 (1).

A few years later biochemical analysis of brain tissue revealed that the acitivity of the L-DOPA producing enzyme tyrosine hydroxylase is diminished in the substantia nigra of parkinsonian patients (2). These findings were confirmed and extended by Riederer and coworkers, showing that tyrosine hydroxylase is reduced not only in the brain but also in the adrenal medulla of parkinsonian patients (3). Furthermore it has long been known that the activity of this enzyme is regulated by catecholamines such as L-DOPA via a feedback inhibition (4, 5); in other words L-DOPA inhibits its own endogenous biosynthesis. This might be the reason why L-DOPA does not work in a number of parkinsonian patients, in particular after long-term treatment. Other therapeutic strategies have to be considered. One approach might be the activation of the endogenous L-DOPA biosynthesis by stimulating the defect tyrosine hydroxylase in parkinsonian patients. As shown by Nagatsu (6), this enzyme is an iron-containing enzyme with tetrahydrobiopterin (H4biopterin) as its coenzyme. H4biopterin was used in clinical trials in the early seventies. Relief of symptoms or improvement in disability could not be observed (W. Birkmayer, unpublished observations). A possible explanation for this therapeutic failure is provided by the finding that H4biopterin crosses the blood-brain barrier very poorly and can therefore not evoke its action in the brain (7). On the other hand it has been shown

that the special iron compound Oxyferriscorbone® is able to improve symptoms of parkinsonian patients (8, 9). This observation makes it likely that tyrosine hydroxylase is stimulated in vivo, because the increase in the patients' mobility is paralleled by an increase in the urine level of homovanillinic acid (HVA) (9, 10).

As shown by Nagatsu (11) H4biopterin is considerably reduced in the brain of parkinsonian patients, and may be the reason for the tyrosine hydroxylase deficiency. This deficiency could be due either to decreased biosynthesis or to a lack of the biologically active form. If the latter is the case, activation of H4biopterin from its precursor H2biopterin should yield a higher tyrosine hydroxylase activity and thus increased endogenous L-DOPA synthesis. As nicotinamide adenine dinucleotide in its reduced form (NADH) is the coenzyme of dihydropteridinereductase, the enzyme which regains H4biopterin from its oxidized precursor (12), we tried this compound medication for parkinsonian patients.

Materials and methods

The diagnosis and disability scores of the Parkinson patients were established according the scale of Birkmayer and Neumayer (13).

β-nicotinamide adenine dinucleotide, reduced from disodium salt abbreviated NADH (Synonyms: β-NADH, reduced DPN, β-DPNH and β-nicotinamide adenine dinucleotide phosphate, reduced form), abbreviated as NADPH, were purchased from Sigma Diagnostics (St. Louis, MO, USA). 25 mg of NADH were dissolved in 100 ml of 0.9 percent sterile sodium chloride, pH 7.4, and intravenously infused for 30 minutes. NADH solution was always prepared freshly immediately prior to use. NADPH infusions were prepared in an identical fashion. Disability scores were determined within four hours after the NADH application.

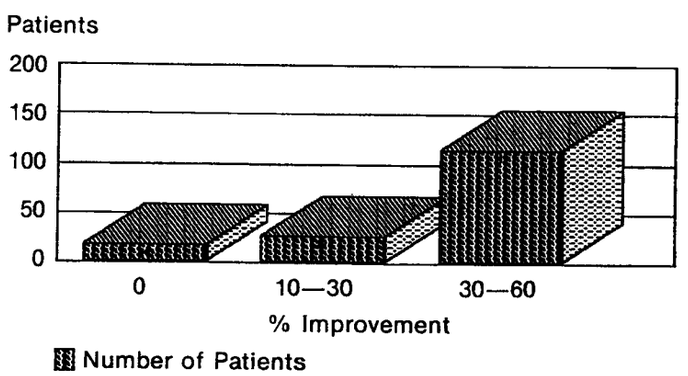
Patients received NADH infusion either every day or every second day, depending on their disability score and their improvement. The treatment was continued for 10 to 14 days. As soon as the patients deteriorated after withdrawal of the NADH medication (usually after 2 or 3 weeks), NADH treatment was started again. The first morning urine was collected for HVA measurements before the beginning of the treatment and the day after clinical improvement was recognized. Patients treated with NADPH received infusion either once or twice a week. Disability scores were examined before and after one week of treatment.

Results

161 patients have been treated with NADH since October 1987 until December 1988.

115 patients (71.4 %) exhibited very good improvement of their disability (better than 30 %) and 28 patients (17.4 %) a moderate response (up to 30 %). 18 patients (11.2 %) did not respond to NADH (Fig 1.). The overall improvement of the disability of the 143 patients was 37.0 ± 18.0. In particular the walking and pushing ability improved considerably and so did posture, speech and mimic. The action of NADH lasted between 1 and 7 days, depending on the severity of the symptoms. At withdrawal of NADH a worsening of the patients' disability was observed after 2 to

Fig. 1. NADH in Parkinson therapy. Improvement in disability



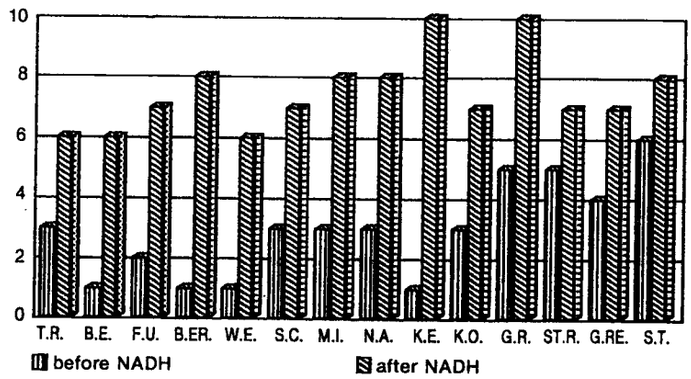


Fig. 2. Prolongation of "on-phases" by NADH

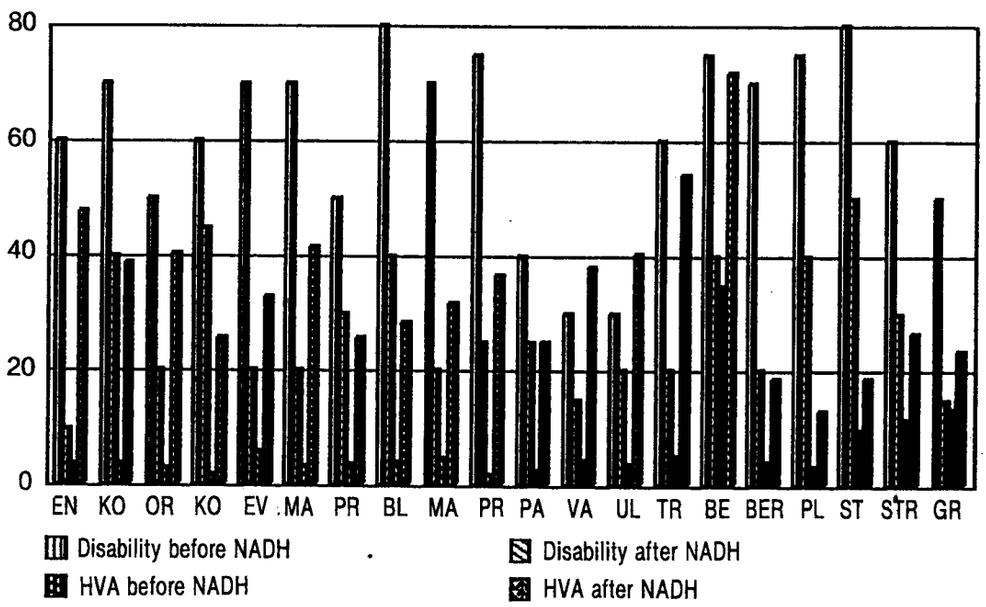
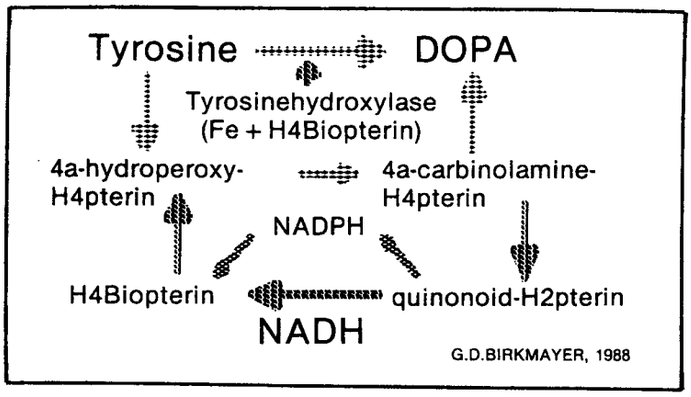


Fig. 3. HVA urine level after NADH treatment

6 weeks. This indicates the improvement of the symptoms to be based on the NADH applied. In addition to the improvement another effect of NADH was observed. Fifteen patients are doing very well with NADH alone. In these cases the usual Parkinson therapy Madopar® could be omitted totally. In the other patients the dosage of L-DOPA could be reduced by up to30 % of the original dose. Fifteen patients have been examined with regard to the duration of the daily "on" and "off phases". The daily on-phases could be increased considerably in the range from 2 to 9 hours (Fig. 2). In twenty-eight patients who exhibited a very good improvement in disability, the urine level of the dopamine metabolite homovanillinic acid (HVA) was determined. In all the patients examined a striking increase of the concentration of HVA in the urine was found (Fig. 3). A similar HVA increase is also observed after treatment with L-DOPA, implying that treatment with NADH causes a comparable effect to that of the standard Parkinson medication L-DOPA, namely an increase in the urine HVA concentration. In other words, NADH may substitute for L-DOPA. As NADH is not a direct precursor of L-DOPA it seems likely that it acts via a stimulation of the endogenous L-DOPA biosynthesis. At the moment we can only speculate on the possible mechanism by which this stimulation of L-DOPA biosynthesis may occur. As shown on the chart of Figure 4, L-DOPA is formed from tyrosine by the enzyme tyrosine hydroxylase. This en-

Fig. 4. Mechanism of NADH action



zyme is an iron-containing enzyme with tetrahydrobiopterin (H4Biopterin) as its coenzyme. H4Biopterin provides electrons to reduce molecular oxygen and is in turn oxidized to quinonoidH2pterin. H4biopterin is restored by quinonoiddihydropteridinreductase (DHPR), an enzyme which reduces quinonoidH2pterin back to H4biopterin. The cofactor of DHPR is NADH (12). It has been found by Nagatsu and coworkers that the levels of H4biopterin in the brain and in the cerebrospinal fluid of parkinsonian patients were reduced to about 50 % in comparison to that of age-matched healthy individuals (11). This H4biopterin deficiency could be due either to decreased biosynthesis or to a lack of the biologically active form of H4biopterin. The coenzyme of the quinonoiddihydropteridinreductase, NADH, seems to be involded in the production of active H4biopterin, which for its part is responsible for the full enzymatic activity of tyrosine hydroxylase. The clinical effect of NADH, which closely resembles that of L-DOPA, supports our assumption of the stimulation of endogenous L-DOPA biosynthesis. Indirect evidence for this stimulation of endogenous L-DOPA biosynthesis has been obtained from the increase in the urine level of HVA after NADH treatment. A comparable increase in the HVA urine level was observed after the standard L-DOPA therapy. Another coenzyme, nicotinamideadeninedinucleotidephosphate (NADPH), was also tried as medication for Parkinson's disease. The reason for this was twofold. The first reason was that 18 patients did not exhibit any beneficial clinical effect after NADH treatment. The second reason was that 2 enzymes exist which can restore the consumed H4biopterin, namely DHPR, already mentioned, and dihydrofolatereductase (DHFR). The co-factor of the latter is NADPH (see also Figure 4). If dihydrofolatereductase (DHFR) is involved in the biosynthesis of H4biopterin, NADPH should show a clinical effect comparable to that of NADH. According to our preliminary data given in Table 1, this seems to be the case. 8 patients have been treated with NADH. All of them exhibited a beneficial clinical effect. 4 patients showed a very good improvement in their disability (better than 30 %), and 3 patients a moderate response (up to 30 %). The detailed data on these 8 patients are given on Table 1. In 2 patients the basis therapy of Madopar® could be omitted totally. These patients are doing very well with NADPH infusion twice a week. In 4 of the other 5 patients the dose of Madopar® could be reduced. With a dose of 25 mg NADPH twice a week as intravenous infusion, we did not observe any side effects. If doses higher than 50 mg are applied at one infusion, one can occasionally observe a drop in the blood pressure. No other side effects have been seen even after a period of treatment of 6 weeks and more.

In order to prove our hypothesis that NADH stimulates the endogenous biosynthesis of L-DOPA in the brain of parkinsonian patients, we have to measure its level before and after NADH treatment in distinct brain areas. With biochemical methods of extracting brain tissue, this goal would be virtually impossible to achivie. Other techniques such as SPEC scan or PET scan may be more promising.

The primary and most important aim for the clinician, however, should always be to help the patients and to improve his symptoms. On the basis of our findings the following therapeutic concept may be conceivable: Restoring the dopamine level by stimulation of its endogenous synthesis

Table I. Data of NADPH patients

Patients	Previous Therapy	New Therapy	Disability before	after
J.U. 44m	Ma 125/1x + Ju/1x	NADPH 25/2x/w	40	15
E.W. 74m	Ma 200/3x/d	NADPH 25/1x/w Ma 125/3x/d	75	30
N.K. 86m	Ma 100/6x/d	NADPH 25/3x/w	70	50
T.J. 86m	Ma 125/1x/d,Ju 2x/d	NADPH 50/2x/w Ma 250/2x/d,Ju 2x/d	70	50
F.K. 80m	Ma 100/3x/d,Ju/2x	NADPH 25/2x/w Ma 125/2x/d	60	15
T.P. 45m	Si 125/6x/d,Ju/2x	NADPH 25/2x/w Si 125/6x/d, Ju/2x	50	15
N.J. 76m	Ma 125/3x/d,Ju 2x/d	NADPH 25/2x/w Ma 62,5/3x/d	70	15
F.K. 80m	Ma 100/3x/d,Ju/2x	NADPH 25/2x/w Si 62.5/3x/d,Ju/2x1/2	60	15

Ma = Madopar® (levodopa + benserazid), Si = Sinemet® (levodopa + karbidopa), Ju = Jumex® (deprenyl), m = male.

by NADH, NADPH or iron and retarding its degradation by monoamine oxidase B-inhibitors such as deprenyl.

Future studies will reveal whether the progression of the disease can be prevented by such a therapeutic regimen.

References

1. Birkmayer W, and Hornykiewicz O. Der L-Dioxyphenylalanin (L-DOPA) Effekt bei der Parkinson-Akinese. Klin Wschr 1961:73:787.
2. McGeer PL, McGeer EG, Wada JA. Distribution of tyrosine hydroxylase in human and animal brain, J. Neurochem. 1971:18:1647.
3. Riederer P, Rausch WD, Birkmayer W. CNS modulation of adrenal tyrosine hydroxylase in Parkinson's disease and metabolic encephalopathies J. Neur Trans Suppl 1978:14:121—132.
4. Nagatsu T, Levitt M and Udenfried S. Tyrosinehydroxylase. The initial step in norepinephine biosynthesis. J Biol Chem 1964:239:2910—2917.
5. Ames MM, Lerner P and Lovenberg W. Tyrosinehydroxylase: activation by protein phosphorylation and end product inhibition. J Biol Chem 1978:253:27—31.
6. Nagatsu T, Yamaguchi T, Rahman K et al. Catecholamine-Related Enzymes and the Biopterin Cofactor in Parkinson's Disease and Related Extrapyramidal Diseases. Advances in Neurology 1984:40:467—473.
7. Curtius HC, Niederwieser A, Levine R and Muldner H. Therapeutic efficacy of Tetrahydrobiopterin in Parkinson's Disease. Adv Neurol 1984:40:463—466.
8. Birkmayer W and Birkmayer JGD. Iron — a new aid in the treatment of Parkinson patients. J Neur Trans 1986:67:287—92.
9. Birkmayer JGD and Birkmayer W. Improvement of disability and akinesia of patients with Parkinson's disease by intravenous iron substitution. Ann Clin Sci 1987:17:32—35.
10. Birkmayer W, Birkmayer GJD. Iron Therapy in Parkinson's Disease. Stimulation of Endogenous Presynaptic L-DOPA Biosynthesis by the Iron Compound Oxyferriscorbone. New Trends in Clin Neuropharmacol. II, 1988:3:185—191.
11. Nagatsu T, Namaguchi T, Kato T, Sugimoto T, Matsura S, Akino M, Nagatsu I, Iizuka R, Narabayashi H. Biopterin in human brain and urine from controls and parkinsonian patients: application of a new radioimmunoassay. Clin Chim Acta 1981:109:305—311.
12. Nichol CA, Smith GK and Duch DS. Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. Ann Rev Biochem. 1985:54:729—764.
13. Birkmayer W and Neumayer E. Die moderne medikamentöse Behandlung des Parkinsonismus, Z Neurol 1972:202:257—280.

Address:
Georg J. D. Birkmayer, M.D.
Birkmayer-Institut für Parkinsontherapie
Schwarzspanierstrasse 15
A-1090 Vienna, Austria