Nicotinamide adenine dinucleotide (NADH) — a new therapeutic approach to Parkinson's disease
Comparison of oral and parenteral application

The reduced coenzyme nicotinamide adenine dinucleotide (NADH) has been used as medication in 885 parkinsonian patients in an open label trial. About half of the patients received NADH by intravenous infusion, the other part orally by capsules. In about 80 % of the patients a beneficial clinical effect was observed: 19.3 % of the patients showed a very good (30—50 %) improvement of disability, 58.8 % a moderate (10—30 %) improvement. 21.8 % did not respond to NADH. Statistical analysis of the improvement in correlation with the disability prior to treatment, the duration of the disease and the age of the patients revealed the following results: All these 3 parameters have a significant although weak influence on the improvement. The disability before the treatment has a positive regression coefficient (t value <0.01). The duration of the disease has a negative regression coefficient (<0.01) and so has the age a negative regression coefficient (t value <0.05). In other words younger patients and patients with a shorter duration of disease have a better change to gain a marked improvement than older patients and patients with longer duration of the disease. The orally applied form of NADH yielded an overall improvement in the disability which was comparable to that of the parenterally applied form.

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Introduction

It is generally accepted that Parkinson's disease is caused biochemically by a deficit of dopamine in the basal ganglia of the brain stem (1). The immediate precursor of dopamine, L-DOPA, is able to substitute the shortage in dopamine (2), whereas tyrosine, a precursor of L-DOPA, is unable to do so. This indicates that the biosynthesis of dopamine is blocked at the metabolic conversion from tyrosine to L-DOPA. The enzyme catalyzing this reaction is tyrosine hydroxylase (TH) the activity of which is considerably diminished in substantia nigra of parkinsonian patients (3, 4).

Indirect evidence for the central role of TH in the biosynthesis of dopamine has been gained long ago by applying α-methylparatyrosine, an inhibitor of TH, to parkinsonian patients. Under this medication the disability of the patients deteriorated indicating a further reduced L-DOPA biosynthesis (5). In 1981 Nagatsu and coworkers (6) showed that H4biopterin, the coenzyme of TH, is reduced to about 50 % in the brain of parkinsonian patients in comparison to that of age matched healthy control. This may be one of the reasons for the reduced TH activity.

For the time being the first choice of therapy is still substitution by L-DOPA which is the end product of

TH and readily converted into dopamine. L-DOPA is known to act as feedback inhibitor of TH (7). Thus the exogenous supplied L-DOPA will inhibit TH activity already reduced in parkinsonian patients even further. Taking this into account we considered a new concept to overcome the dopamine deficit namely to stimulate TH activity in order to increase L-DOPA biosynthesis. This may be accomplished by adding the reduced or missing co-factors. Since we know that the coenzyme of TH H4biopterin is also reduced in the brain of parkinsonian patients, therapeutic application of this substance was considered. However, clinical trial with H4biopterin did not show any beneficial clinical effect with Parkinsonian patients (8, 9). The failure of this approach was the impermeability of the blood brain barrier for H4biopterin. Therefore, this substance cannot reach its target, the substantia nigra in the brain. The question was whether it is possible to stimulate the H4biopterin biosynthesis in the brain. The H4biopterin deficiency could be due either to a decreased biosynthesis or to a lack in the biological active form. If a diminished biosynthesis of H4biopterin is the cause of TH defect, stimulation of H4biopterin biosynthesis should elevate the enzyme activity. The key enzyme in H4biopterin biosynthesis is the quinoidH2pteridin reductase (10). This enzyme

needs the reduced nicotinamide adenine dinucleotide (NADH) as coenzyme. Our idea was to stimulate H4biopterin biosynthesis by applying NADH which increases the quinonoidH2pteridine reductase activity and due to this the amount of H4biopterin increases. Owing to this NADH may stimulate endogenous L-DOPA biosynthesis by the postulated mechanism. An increase in L-DOPA production should be reflected by an improvement of the clinical symptoms of parkinsonian patients. In order to investigate our concept in more detail more than 800 parkinsonian patients have been treated with NADH and the possible mechanism of action of NADH has been studied in a dopamine producing neuroblastoma cell-line.

Materials and methods

In an open label trial 885 parkinsonian patients have been treated with the coenzyme. Diagnosis and disability scores of parkinsonian patients were established according to the scale of Birkmayer and Neumayer (11).

NADH (synonymns: β-NADH, Reduced DPN, β-DPNH) was obtained from Boehringer Mannheim. 12.5 mg of NADH were dissolved in 100 ml 0.9 percent sterile sodium chloride, buffered, pH 7.6, filtered through a 0.22 micron Milipore filter and intravenously infused in 30 minutes. NADH solutions were prepared always fresh immediately prior to use. One group of patients received NADH infusion every other day. The other group of patients obtained 5 mg NADH orally in form of capsules. Treatment was given every other day for 14 days. The disability score was determined before the first NADH treatment and after the last NADH treatment which was in most cases 14 days from the beginning of the therapy.

Results

885 patients were included in this study. 42 patients (4.7 %) showed a 50 % improvement in disability, 54 (6.1 %) a 40 % improvement, 75 (8.4 %) a 30 % improvement, 147 (16.6 %) a 20 % improvement and 374 (42.2 %) a 10 % improvement (Fig. 1). 193 (21.8 %) did not respond to NADH.
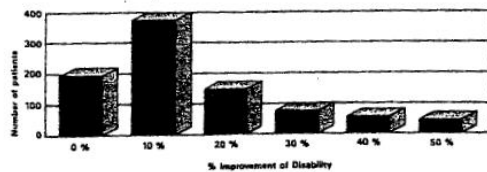

Fig. 1. Improvement of disability by NADH therapy.

When the 415 patients receiving NADH intravenously were compared with those 470 persons getting the oral form of NADH the mean value of improvement in disability after i.v. applied NADH was 20.6 % after orally applied NADH 19.8 % (Table 1). These findings indicate that the oral form of NADH shows a beneficial clinical effect comparable to that of the i.v. applied NADH not only in the mean value but also in the maximum value. The maximum improvement of i.v. applied NADH was 55 %, the maximum of orally applied NADH 60 %. The motoric ability improved considerably in these patients, in particular the walking, pushing, posture and speech as well as mimic.

After withdrawal of NADH from the usual medication, a worsening of the patient's disabilities was observed in between 2—3 weeks. This indicated the improvement of the symptoms to be caused by the NADH applied. In order to substantiate whether NADH is able to stimulate L-DOPA biosynthesis we tested this substance in tissue culture. When neuroblastoma cells were incubated with NADH a dosage dependent increase in dopamine production was observed (Fig. 2). In the presence of 200 µg NADH a four fold increase in dopamine production was observed. This stimulation was independent from the tyrosine supplied exogenously indicating that the substrate tyrosine is not the limiting factor but the enzyme or the coenzyme, respectively.

The stimulation of dopamine production increased with the number of cells. In the presence of 200 µg of NADH/ml 20 million cells yielded 12 ng of dopa-

Table 1. Improvement of disability by NADH given orally or intravenously (i.v.)

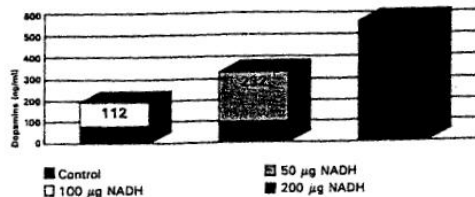| Variable	N	Mean	SEM	SD	Min.	Max.	Median
Disability NADH i.v.	415	58.6	0.8	16.5	15	90	80
Improvement NADH i.v.	415	20.6	0.8	12.6	-15	55	20
Disability NADH oral	470	56.5	1.0	18.9	15	85	80
Improvement NADH oral	470	19.8	0.8	12.4	-10	60	20

Fig. 2. A concentration dependent increase of dopamine production in neuroblastoma cells caused by NADH.

mine/ml, 40 million cells 44 ng dopamine/ml and 60 million cells 72 ng despectively. (Fig. 3),

When TH activity was measured directly after NADH had been added to the culture medium, a 75 % increase could be observed (Fig. 4). This finding indicates that NADH is able to stimulate TH activity directly.

A positive correlation between age and disability before treatment as well as between duration of the disease and disability before treatment appears to be most plausible.

According to this one might expect that both high age and long duration of the disease coincide with a marked improvement. However, a refined statistical analysis ends up with the contrary, meaning that a negative correlation between age and improvement of the treatment as well between duration of the disease and improvement after treatment is obtained. For an accurate assessment of the real relationship between these variables (disability before treatment, age, duration of disease and improvement) it is necessary to substract the effects of the variable disability before treatment. The results of this calculation show that in general younger patients and patients with a shorter duration of disease respectively have a better chance to gain a marked improvement than older patients and patients with a longer duration of the disease.

Discussion

This study confirms and extends our previous reports on the clinical benefit of NADH for parkinsonian patients (12, 13, 14). The new finding of this report is that the oral form of NADH shows a beneficial clinical effect comparable to that of the intravenously applied NADH. The galenic formulation of the oral
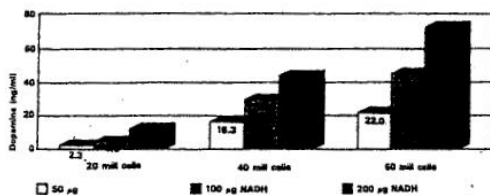

Fig. 3. Effect of the number of neuroblastoma cells on the increase of dopamine production caused by NADH.
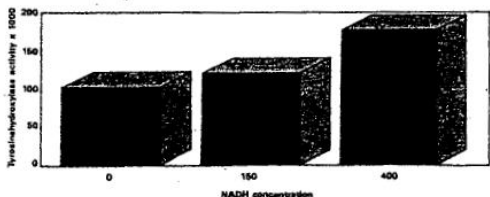

Fig. 4. Stimulation of tyrosine hydroxylase activity by NADH.

form of NADH is a critical factor with regard to its clinical efficacy. When we first used NADH filled in gelatine capsules the effect was not convincing. This was most likely due to the rapid dissolution (approximately 10—15 minutes) of the capsules leading to a release of NADH into the acid condition of the stomach. Since NADH is rapidly oxidized below pH 7.6 the conditions in the stomach will inactivate NADH by converting it to NAD+. The investigations of this report were therefore performed with NADH capsules coated with an acid-stable film and a release time of 2—3 hours. With this galenic formulation of NADH an improvement in disability could be achieved which was comparable to that of intravenously applied NADH. It should be pointed out that most of the patients included in this study received in addition to NADH the classical medication such as Madopar® or Sinemet® with or without addition, such as deprenyl, bromocryptine or amantadine. In many of these patients the daily dose of L-DOPA could be reduced considerably. In some patients it could be omitted totally.

The question is whether or not the well established L-DOPA therapy should be replaced by NADH treatment. Arguments in favour of the new NADH treatment become apparent when the biochemical and pharmacological differences behind these 2 therapeutic concepts are considered. The L-DOPA therapy follows the principle of substitution meaning that dopamine deficit is filled up by substituting with its immediate precursor L-DOPA. However, as we know, substitution of certain biological substances by exogenous supply will lead to a depression of the organism's own biosynthesis. This holds for cortisone, thyroxine, aldosterone, many other hormones and metabolic substances. It is certainly valid also for L-DOPA biosynthesis. In other words exogenous supply of L-DOPA will inhibit its endogenous biosynthesis. As already mentioned the L-DOPA producing enzyme, TH, is considerably reduced in parkinsonian patients (3, 4). It is also known that TH is inhibited by its end product L-DOPA (7, 15). This implies that TH working already insufficiently is further inhibited by the exogenous supplied L-DOPA. A further reduction in enzymatic activity will be the consequence. Whether or not this is the cause of the frequent observed "off"-effect, in particular after long-term treatment with L-DOPA, remains to be elucidated. The NADH therapy on the other hand follows an opposite strategy, namely the stimulation of the endogenous L-DOPA biosynthesis by activation of the key enzyme TH. There are a number of arguments in favour with NADH treatment one of which is that patients which do not respond to the classical L-DOPA therapy even if higher dosages are applied, show an improvement after NADH treatment. The stimulation

of L-DOPA biosynthesis may occur via enhanced production of the TH coenzyme H4biopterin. As shown by Nagatsu and coworkers the levels of H4biopterin in the brain and cerebrospinal fluid of Parkinsonian patients are reduced about 50 % in comparison to that of age matched healthy individuals. The cause of this H4biopterin deficiency is still obscure. If the deficit in H4biopterin is due to a decreased biosynthesis, the biochemical mechanism of the NADH action may be explainable. H4biopterin is formed from H2pterin by an enzyme called quinoiddi-hydropteridinreductase (DHPR), an enzyme which needs NADH as essential cofactor (10). There is indirect evidence that DHPR influences TH activity via H4biopterin biosynthesis because substances which competitively inhibit DHPR such as 1-methyl-4-phenyl- 1,2,3,6 tetrahydropyridine (MPTP) induce parkinsonian symptoms (16). A valid argument for clinical efficacy of NADH as stimulation of L-DOPA biosynthesis would be the measurement of an increase of L-DOPA concentration in the brain, in particular in substantia nigra. For obvious reasons it is impossible to gain these data because nobody, for the time being, is able to measure the L-DOPA concentration in the substantia nigra directly before and after NADH treatment. Therefore we have to rely on indirect evidence one of which is the metabolic product of dopamine and homovanillinic acid (HVA) respectively. The level of this substance increases after NADH treatment parallel to the improvement in disability. Furthermore by tissue culture experiments using dopamine producing neuroblastoma cells we were able to show that NADH is able to increase the production of dopamine. Furthermore NADH does stimulate the enzymatic activity of TH directly when added to the culture medium. These findings indicate that NADH acts directly on TH and due to this stimulates dopamine biosynthesis which is a considerable support for our clinical concept of stimulation the endogenous dopamine production. It may be argued that the beneficial clinical effect observed under NADH medication is not a central nervous system related but a peripheral one. If this is actually the case an increase in L-DOPA in the blood will be the consequence. From this amount a certain percentage will reach the brain by potentially the same mechanism by which exogenously supplied L-DOPA reaches the brain. Indirect evidence for this assumption is derived from the observation that a DOPA decarboxylase inhibitor such as carbidopa, given to a number of patients in combination with NADH yielded a better and longer lasting clinical improvement than NADH alone.

The new therapeutic principle for treating Parkinson's disease, namely the stimulation of the endogenous L-DOPA biosynthesis, could overcome the drawback of the L-DOPA treatment in the sense that it could avoid further destruction of the residual nigra cells caused by the action of radicals which are formed in considerable quantities by autooxidation of L-DOPA. A double-blind study is in progress in our Institute which will provide a definite conclusion about the clinical efficacy of this essential autosomal substance as new therapeutic concept for Parkinson's disease.

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