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EVALUATION OF ELECTROPHYSIOLOGICAL AND CLINICAL TESTS IN AN EXPLORATORY TRIAL OF ORG 2766 IN MOTOR NEURON DISEASE

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Abstract—Twenty four patients with motor neuron disease (MND) participated in a double-blind, placebo-controlled trial with the ACTH 4–9 analog, Org 2766. Patients were examined three times during an 8 week treatment period, using a summated score for several manually and functionally tested muscles (sum score), myometry, jitter, fibre density (FD), macro motor unit potential (MUP), and supramaximal evoked muscle action potentials. No differences were found between Org 2766 and placebo treated patients. In an open 1 yr follow-up study, 5 out of 13 patients treated with Org 2766 died; the others showed continued progression of weakness. The methods used for assessment of muscle function were compared. The highest intertest reliability was obtained in the sum score and myometry. Mean differences that might be detectable were relatively small for the sum score and myometry, and large for FD and MUP. We concluded that clinical function testing and myometry are superior to electromyographic measurements for assessment of changes in MND patients.

Key words: ALS, Org 2766, treatment, evaluation methods.

INTRODUCTION

Small exploratory trials in patients suffering from motor neuron disease (MND) (amyotrophic lateral sclerosis and progessive spinal muscular atrophy) are frequently performed to study possible influences of medication on the course of the disease. To date nearly all of these trials have been negative [1]. The effect of medication is usually investigated by measuring muscle strength, either manually or with a myometer [2]. A drawback of these methods is that they measure the sum-result of de- and re-innervation; the results may be normal although a considerable number of motoneurons have been lost. In addition, these measurements are influenced by the patients' motivation [3]. As an alternative, neurophysiological measurements of the process of de- and re-innervation itself, might be considered. The question is, however, whether the results obtained by electromyography are sufficiently reproducible and sensitive to be of value in longitudinal investigations.

The ACTH 4–9 analog Org 2766 has been shown, in previous experimental investigations, to enhance regeneration of axons after nerve lesions, and to stimulate collateral sprouting and re-innervation after partial denervation of a rat skeletal muscle [4–9]. It also protects from neurotoxicity in rat and human [10, 11]. We performed a double-blind, placebo-controlled exploratory trial of Org 2766 in MND patients, which was followed by an open trial; no effect could be demonstrated. The opportunity was used to investigate whether electromyographic tests were to be preferred to clinical measurements in the assessment of muscle function in MND trials.

PATIENTS AND METHODS

Patients

Patients included in this study were selected from the MND patients visiting the out patient clinic for neuromuscular diseases in the University Hospital Utrecht. All patients who

Table 1. Muscle functions tested to determine sum score*

Bulbar functions	Facial muscles Speech Mastication Swallowing Coughing
Trunk muscles	Lifting the head Respiration Raising from supine to sitting position Turning from supine to prone position Straightening from stooping position Standing up from crouching position
Upper limbs (bilaterally)	Abduction of the upper arm Extension and flexion of the arm at the elbow Extension and flexion of the fingers
Lower limbs (bilaterally)	Flexion of the thigh Flexion and extension of the leg at the knee Dorsal and plantar flexion of the feet

*Adapted from [16].

met the selection criteria and gave informed consent were admitted to the study.

Inclusion criteria. (a) Age between 30 and 70 yr; (b) clinically progressive muscle weakness accompanied by muscle atrophy; (c) no sensory symptoms; (d) evidence of denervation and re-innervation on concentric needle electromyography, with fibrillation potentials, positive sharp waves and large amplitude motor unit potentials in at least three limbs, or in two limbs and a bulbar muscle; (e) no pyramidal signs in the lower limbs. Patients with pyramidal signs in the lower limbs were excluded from the trial, in view of the intended neurophysiological studies (see below); (f) normal or slightly decreased (less than 30%) motor nerve conduction velocity and normal sensory nerve conduction velocity; (g) serum creatine kinase activity not above 300 U l^{-1}; (h) prior to admission to the trial, patients were submitted to an intake electrophysiological investigation. Patients were only admitted when, at the time of intake, the mean jitter in the anterior muscle was between 50 and 90 μsec. This limitation was chosen in view of observations in a previous pilot study [12], to select patients in whom both improvement and deterioration of electromyographic changes could take place.

Exclusion criteria. (a) Cerebrospinal fluid examination, myelography, and CT scanning were performed when indicated. Patients were excluded when these investigations revealed another possible cause for the patients' symptoms; (b) patients were excluded when haematological, biochemical and serological studies revealed other possible causes for the patients' symptoms; (c) signs of malignancy on a thorax X-ray was a reason for exclusion; (d) possible radicular abnormalities in the lower limbs, revealed by prolonged latency or absence of the H-reflex of the soleus muscle were reasons for exclusion; (e) except for the medication described in this trial, patients received no medication with possible influence on MND.

Study design

Double-blind trial. The study was designed as a randomized, double-blind, placebo-controlled trial, lasting, in each case, for 8 weeks. This time period was chosen because it was assumed to be of sufficient length for an increased sprouting reaction to occur in Org 2766 treated patients. Effects of Org 2766 medication on intramuscular sprouting and muscle function are seen within weeks in laboratory animals [9]. Observations in critically ill patients have shown that humans are able to produce large numbers of collateral sprouts within 3 weeks [13]. The capacity for sprouting of intramuscular nerve fibres in MND is not lost [14], although it appears to be diminished [15]. Little change due to disease progression can be expected in a period of 8 weeks. Inclusion criteria, methods for patient evaluation and medical treatment were specified in a protocol and approved by the ethical committee of the University Hospital Utrecht. Patients entered the study on the basis of informed consent. They were injected subcutaneously at random with either placebo, a low dose

Table 2. Changes in clinical and electrophysiological values

	(N)	Start trial	After 4 weeks	After 8 weeks	P_{time}	$P_{treatment}$
		Mean (± standard error of mean)				
Sum score (max. score = 69)						
All patients	(24)	57.1 (1.6)	55.7 (1.8)	54.3 (2.0)	0.001	
Placebo	(8)	55.3 (3.2)	54.5 (3.3)	53.5 (3.5)		
Low Org 2766	(7)	58.5 (2.9)	58.1 (3.7)	57.4 (4.3)		0.22
High Org 2766	(9)	57.6 (2.3)	54.9 (3.0)	52.8 (3.3)		
MVIC (normal mean value = >250 Newton*)						
All patients	(24)	144 (7)	134 (6)	136 (6)	0.21	
Placebo	(8)	141 (13)	126 (13)	135 (12)		
Low Org 2766	(7)	139 (12)	135 (12)	138 (11)		0.72
High Org 2766	(9)	150 (12)	140 (9)	137 (11)		
Jitter (normal mean value 32.1 μs†)						
All patients	(24)	73.7 (5.5)	75.9 (4.2)	75.7 (4.4)	0.76	
Placebo	(8)	87.9 (12.5)	89.7 (7.5)	79.7 (9.9)		
Low Org 2766	(7)	67.8 (9.9)	71.7 (7.8)	76.7 (9.7)		0.16
High Org 2766	(9)	65.6 (4.5)	66.9 (4.8)	71.3 (3.9)		
FD (normal mean value 1.53–1.73†)						
All patients	(24)	2.27 (0.07)	2.53 (0.11)	2.49 (0.09)	0.05	
Placebo	(8)	2.42 (0.15)	2.68 (0.31)	2.60 (0.20)		
Low Org 2766	(7)	1.98 (0.07)	2.56 (0.12)	2.41 (0.16)		0.28
High Org 2766	(9)	2.37 (0.10)	2.36 (0.10)	2.46 (0.14)		
MUP area (normal median value = 800–1050 μV·ms‡)						
All patients	(20)	2740 (330)	2650 (200)	2540 (260)	0.61	
Placebo	(6)	2690 (470)	2900 (360)	2450 (350)		
Low Org 2766	(6)	2650 (510)	2320 (220)	2700 (460)		0.69
High Org 2766	(8)	2840 (710)	2700 (380)	2600 (530)		
SEMAP area (normal median value = 15,000–85,000 μV·ms §)						
All patients	(16)	38,450 (4150)	39,540 (5470)	36,780 (3790)	0.60	
Placebo	(15)	33,270 (4680)	45,160 (14,930)	37,610 (8230)		
Low Org 2766	(4)	38,200 (12,650)	40,740 (6950)	40,870 (6400)		0.08
High Org 2766	(7)	42,330 (4400)	34,850 (6590)	32,450 (5250)		

N = the number of patients; P_{time} = significance level for changes in time; $P_{treatment}$ = significance level for differences between treatment groups in trends in time; * see [22]; † see [23]; ‡ see [24] and [25]; § see [21].

Table 3. Reliability of variables and sensitivity to changes

	Cronbach's α	Intra-individual S.D.	Minimal mean difference detectable $\alpha=0.05$, $\beta=0.20$, $N_1=12$, $N_2=12$	Mean difference between MND patients and controls	Ratio*
Sum score	0.97	2.3	2.75	13.3	0.21
MVIC (N)	0.88	17	20.3	106	0.19
Jitter (μs)	0.91	11.2	13.4	43.0	0.31
FD	0.63	0.37	0.44	0.81	0.54
MUP (μV·ms)	0.88	664	694	1639	0.42
SEMAP (μV·ms)	0.85	9230	11,041	32,850	0.34

Ratio* = the detectable change with $\alpha=0.05$, $\beta=0.2$ and total $N=24$, divided by the mean difference between MND patients and controls. This gives an indication of the relative effect a drug must have on the variable, before it will become apparent in an experimental study.

Org 2766 (0.25 mg m^{-2} body surface) or a high dose Org 2766 (2.5 mg m^{-2} body surface) every 48 h. Both were generously supplied by Organon International B.V. (Oss, The Netherlands). At the beginning of the trial, after 4 weeks and after 8 weeks, patients were subjected to a battery of physical and clinical neurophysiological measurements (see below). These were always performed by the same neurologist and neurophysiologist, without reference to previous examinations.

Open trial. At the end of the double-blind trial, patients were offered the opportunity of entering a 1 yr, open trial in which they received Org 2766 injections at a dose of 2.5 mg m^{-2} body surface every 48 h. Patients entering the open trial visited the out patient clinic every 3 months and underwent a routine physical examination, which included a sum score (see below).

Measurements

Sum score. On each examination day, a

physical examination was performed. The strength of 23 bulbar, trunk, arm and leg muscles was tested functionally or manually (Table 1). Assessment of the function of bulbar and trunk muscles was performed with the methods described by Louwerse *et al.* [16]. The strength was scored as either 3 (normal), 2 (slightly decreased), 1 (greatly decreased) or 0 (no visible muscle contraction or function). The scores were summated and gave a maximal "sum score" of 69.

Myometer-measured maximum voluntary isometric contraction (MVIC). MVIC was measured in one tibial anterior muscle. MVIC was measured using a handheld electrodynamometer [17] (Penny and Giles Ltd, Dorset, U.K.) applied just proximal to the metatarsophalangeal joints at the dorsal surface. Measurements were repeated three times and the mean value was noted as the strength of that tibial anterior muscle.

Neurophysiological examination of the tibial anterior muscle included jitter, fibre density (FD), macro motor unit potential (MUP) and supramaximal-evoked muscle action potential (SEMAP). The measurements were all performed in the same tibial anterior muscle as used for MVIC. They were recorded at different depths of the tibial anterior muscle using three skin insertions, equally spaced, perpendicular to the direction of the muscle fibres. Measurements were always made at a distance of approximately 2 cm from the estimated endplate zone. A 4 cm long, 0.55 mm diameter needle electrode was used for recording (Medelec SFM 37.53114) on a Dantec Counterpoint Apparatus, via two channels. One channel (filter setting HP: 500 Hz, LP: 10 kHz) was used to record jitter and fibre density; the other (filter settings: HP: 10 Hz, LP: 5 kHz) for macro EMG. All recordings were made with slight muscle activation. A complete neurophysiological examination required about 1 h.

Jitter. Computer analysis of jitter was performed as described by Stålberg and Trontelj [18]. The mean consecutive difference (MCD) in interpotential intervals was calculated for each of at least 20 pairs of voluntarily activated muscle fibre action potentials at each examination. The mean of the MCD values was used as the value for neuromuscular jitter at that examination.

FD. FD measurements were performed as described previously [18], using a minimum of 20 measurements to calculate the mean FD.

MUP. Macro-EMG was performed as described by Stålberg [19, 20] (for specifications see [21]). For each investigation the aim was to obtain 15 different MUPs. The median area of the MUPs was used as the value of the MUP for that study.

SEMAP. For recording the SEMAP, the deep peroneal nerve was stimulated supramaximally near the head of the fibula by means of monopolar needle electrodes. Electrical stimuli were delivered by a constant current stimulator and consisted of rectangular pulses of 0.2 ms duration. The SEMAP was recorded on the macro-electrode with the electrode in the middle of an insertion channel. A ground electrode was placed between stimulation and recording electrodes [21]. The mean area of three SEMAPs was used as the measure of the SEMAP for that study.

Statistics

Changes from baseline, and differences between treatment groups, were evaluated using the multivariate analysis of variance for repeated measurements as provided in the statistical package for social sciences (MANOVA, SPSS, Chicago, IL, U.S.A.), with the treatment group as between subject factor and the repeated measurements as within subject factor. The sum score (an ordinal scale) was also tested by means of the non-parametric Friedman two-way ANOVA (SPSS, Chicago, IL, U.S.A.). Effects were considered significant if $P<0.05$. To test the changes during the 1 yr open trial, the Student's t-test for paired measurements was applied. For testing the reliability of the measurements we used Cronbach's α for reliability. Intra-individual variances and standard deviations were estimated from the reliability computations (RELIABILITY, SPSS).

RESULTS

Of the 24 patients entering the study, 9 were female and 15 were male. They were aged between 33 and 69 yr (mean 56.4 yr). Fourteen patients only had symptoms of lower motor neuron disease, whereas ten patients also had signs of upper motor neuron disorders at the bulbar or upper extremities level. The 24 patients were divided at random into 3 treatment groups. Eight patients received placebo injections, seven patients received the low Org 2766 dose and nine

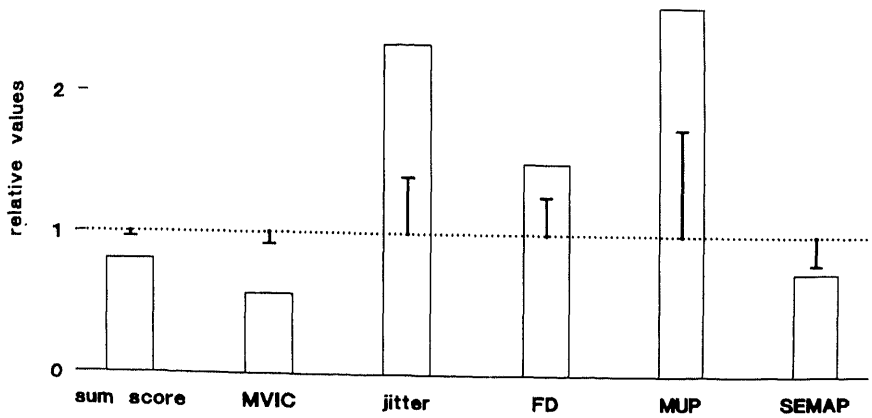

Fig. 1. Schematic representation of usefulness of measurement methods. Mean normal value (corrected for age) in controls is standardized to 1 (dotted horizontal line). Bars represent relative mean values of measurements in MND patients. Vertical standard error lines represent minimal mean difference detectable with measurement methods in a study with 2 groups of 12 MND patients. Usefulness of a measurement method in future trials depends on the ratio between the minimal detectable difference and the mean difference between MND patients and controls.

patients received the high Org 2766 dose. The groups did not differ with regard to age and duration of symptoms: mean age ± standard error of mean (S.E.M.) of the placebo, low dose Org 2766, and high dose Org 2766 groups were 58.9 ± 3.53, 58.1 ± 3.2 and 52.9 ± 3.7 yr, respectively. Mean duration of symptoms was 15.0 ± 2.3, 17.4 ± 1.2 and 21.2 ± 4.2 months, respectively.

Effect of Org 2766

At the beginning of the trial there were no statistical differences between the three groups receiving placebo, low dose or high dose Org 2766, for any of the measurement methods used, nor did any differences develop during the trial (Table 2).

Of the 24 patients who participated in the double-blind trial, 13 entered the open trial and used Org 2766 for 1 yr. Eleven patients refused to enter the open study for various reasons. Of the 13 patients entering the long term study, 5 died from respiratory insufficiency during the observation period of 1 yr. All eight patients alive after 1 yr showed disease progression. The mean sum score of the eight surviving patients at the start of the open trial was 57.6 ± 8.6, and it decreased to 49.2 ± 13.4 ($P<0.01$) after 1 yr. Of the 11 patients not included in the 1 yr follow-up study, 1 patient terminated his life, 4 patients died from respiratory insufficiency, and 6 were still alive at the time of evaluation. No patients were lost to follow-up. As admission to the open study was not random, the number of patients still alive after 1 yr follow-up cannot be used for comparison.

No adverse reactions were reported in any of the treatment groups.

Evaluation of measurements

Since no differences were found in the three treatment groups, these were combined for analysis of the measurement methods (Table 2). Mean values ± standard error of mean are given in Table 2. During the 8 weeks of the study the sum score decreased significantly ($P<0.001$) and the FD increased significantly ($P<0.05$).

Comparison of measurement methods (Table 3)

The reliability of the methods, as calculated by Cronbach's α, was acceptable: 0.85 – 0.97. Only the α for the FD was lower: 0.63. A statistical power analysis was performed to suggest the most promising method for future MND trials. The size of the mean differences which could be demonstrated in this future trial was computed, using the intra-individual variances of the variables, with an α of 0.05, a β of 0.2, and a drug and a placebo group, each consisting of 12 patients. The results of these calculations are given in Table 3. The detectable change is

compared with the mean deviation from normal found in the MND patient group. This deviation is considered to be an indication of the maximal effect which can be achieved by medication. The table suggests three groups of variables: sum score and MVIC will yield significant results with a change of about 20% of the deviation from normal, jitter and SEMAP with a change of about 30% but the FD and macro-MUP require a change of about 50%.

DISCUSSION

In the present double-blind study, using both clinical and neurophysiological measurements, no effect of Org 2766 treatment was found. Furthermore, during 1 yr of treatment with Org 2766, the patients' condition continued to decline. Obviously in the doses used, Org 2766 does not halt disease progression.

During the 8 week trial period, we found no change in strength of the tibial anterior muscle. Nor did we find a change in jitter. Fibre density, however, showed a tendency to increase. This indicates a semi-steady state situation, in which most of the denervation is compensated by re-innervation from still functioning motor neurons. An increase in MUP, which one might expect simultaneously with an increase in fibre density, was not found. It is known, that in the early stages of MND, an increase in FD is found before an increase in MUP amplitude [25, 26]. Furthermore, in the later stages of the disease, Stålberg reported a decrease in the MUP, while the FD was still increased [27, 28]. He suggested that this was due to a deterioration in the motor unit, with local re-innervation in some areas and drop-out of fibres in other areas. MUPs only decrease when the number of motor units has dropped to 5% or less of the normal complement [29].

In clinical trials for motor neuron disease, manual muscle testing [30] and functional scales [30–32] are most commonly used. We compared the commonly used clinical (i.e. functional and muscle strength) tests, with several neurophysiological tests. A test is considered more useful for follow-up of patients in a trial, firstly, if there is a relatively large difference between the patient group and the normal controls, and secondly, if the variable can be measured reliably so that relatively small differences between treatment groups can be detected (see Fig. 1). The clinical tests (sum score and MVIC) were calculated to be most sensitive to differences between groups (Table 3). This finding cannot be explained by low inter-test reliability, as α in Cronbach's test was satisfactory for all tests, FD excepted. The low inter-test reliability of the FD may be partly due to the fact that the FD varied little between our patients, and so the variation within a patient was relatively large compared with the inter-patient variance. Another explanation for the superiority of clinical tests, may be that the electrophysiological measurements do not accurately reflect the overall degree of changes in a muscle. They simply depict changes in a limited part of the muscle, while clinical measurements give an indication of the total muscle (namely MVIC) or even a number of muscles (namely sum score). If clinical measurements can demonstrate smaller changes in degree of disease compared to neurophysiological tests, they are likely to be more useful for testing drug effects in long term studies. In pilot studies of a short time period, however, changes in strength might be preceded by changes in neurophysiological parameters. In that case neurophysiological measurements might still be the only ones that can demonstrate an effect of the drug although the effect has to be relatively large before it is detectable.

It is concluded that neurophysiological measurements contribute little to the evaluation of progression of motor neuron disease in long term follow-up studies, compared with the usual measurements of strength.

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