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Randomized double-blind placebo-controlled trial to evaluate the effect of the $ACTH_{4-9}$ analogue ORG 2766 in IDDM patients with neuropathy

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Summary In this study we report a randomized double-blind, placebo-controlled trial to evaluate the effect of ORG 2766 in IDDM patients with peripheral neuropathy. Sixty-two patients were selected based on the following criteria: abnormal vibration perception threshold above the 95th-percentile adjusted for age and/or abnormal warm temperature threshold, both measured in the right hand. The patients were randomized into two treatment groups after baseline studies: Group 1 was treated with placebo and Group 2 was treated with 3 mg of the $ACTH_{4-9}$ analogue ORG 2766 every 24 h. The total study period was 1 year. After 1 year of treatment there was a significant improvement in vibration threshold in Group 1 compared to Group 2. No other parameters improved in the study period. The number of patients selected may have been too small to detect a more important treatment effect. We conclude from this study that ORG 2766 can improve vibration threshold, indicating large myelinated fibre function, but does not affect any the other neurophysiological function tests. [Diabetologia (1994) 37: 408–413]

Key words $ACTH_{4-9}$ analogue, diabetic neuropathy.

Diabetic neuropathy is a frequently occurring complication of IDDM and NIDDM [1, 2]. Patients with sensorimotor neuropathy can be troubled by symptoms of burning or shooting pain and paraesthesia; at a later stage disturbances of motor function can develop [3–5]. The pathogenic factors causing diabetic neuropathy are probably complex: increased flux via the polyol pathway causing myo-inositol deficiency [6–10], oxidative stress related to chronic hypoxia [11–13] and glycation of myelin proteins have all been implicated in the aetiology of experimental diabetic neuropathy [14, 15].

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; ACTH, adrenocorticotropic hormone; SNCV, sensory nerve conduction velocity; MNCV, motor nerve conduction velocity

Treatments for diabetic neuropathy have been largely unsuccessful. Several studies have been conducted in which aldose reductase inhibitors were used: some studies did not show a beneficial effect [16–19], whereas others demonstrated some improvement in MNCV and paraesthetic symptoms [20, 21]. Gangliosides are also reported to be useful in the treatment of diabetic neuropathy [22, 23]. A novel approach is the use of neuropeptide hormones. It has been shown that the $ACTH_{4-9}$ analogue ORG 2766 is effective in the treatment of cis-platinin induced neuropathy, both in animals [24, 25] and in patients with ovarian cancer [26]. We have also demonstrated that ORG 2766 is effective in preventing experimental neuropathy in the streptozotocin-induced diabetic rat [27] and is partly effective in ameliorating existing neuropathy both in the streptozotocin-induced diabetic rat [28] and in the BB/Wor rat [29].

To investigate the potential of ORG 2766 in the treatment of diabetic neuropathy, we carried out a randomized, double-blind, placebo-controlled study in 62 IDDM patients.

Subjects and methods

Subjects

Sixty-two patients with IDDM were recruited in a single centre, the University Hospital Utrecht. The clinical characteristics of the patients are given in Table 1. Inclusion criteria were age 20–65 years, male or female, IDDM (C-peptide negative) and established clinical neuropathy according to the following criteria: abnormal vibration perception threshold above the P95-value (above the 95th-percentile adjusted for age) or abnormal temperature threshold for warmth, both measured at the right hand. Patients with and without neuropathic symptoms were included. Patients were excluded for the following reasons: other causes of neuropathy, body mass index of more than 30 kg/m^2, endocrine diseases, malignancy, excessive alcohol intake, abnormal liver or renal function tests, poor metabolic control of diabetes, arterial insufficiency of the legs and neuropathic foot ulcerations. Informed consent was obtained from all patients and the study was approved by the ethical committee of the Utrecht University Hospital. The study was conducted in accordance with the Declaration of Helsinki.

Metabolic control

All patients were seen by the same physician (B.B.) every 2 months in the out-patient clinic. Patients monitored their own blood glucose concentrations (Haemoglucotest 1–44R, Boehringer Mannheim, Mannheim, Germany) and adjusted their insulin requirements to maintain the blood glucose concentration between 5 and 10 mmol/l. The objective was to keep the HbA_1 concentration stable, avoiding improvement or worsening of metabolic control throughout the study.

Design

Patients were randomized into two treatment groups after baseline measurements had been recorded: Group 1 was treated with placebo and Group 2 was treated with 3 mg of the $ACTH_{4-9}$ analogue ORG 2766 every day. The study period was 1 year. Randomisation was carried out by assigning the patients a number from 1 to 62 as they entered the study. The placebo and ORG medication for the two treatment groups was numbered randomly using a computer programme, which chose random numbers in such a way that the two medications were evenly distributed. The following neurophysiological parameters were measured at the start of the study (baseline) and after 4, 8 and 12 months: (1) vibratory perception threshold. (2) temperature perception threshold for warmth and cold. (3) cardiovascular autonomic tests. (4) nerve conduction velocity (ulnar, tibial and sural nerve). (5) Hoffmann reflex latency. (6) pupillary light reflex studies. These parameters reflect the function of large myelinated nerve fibres, small thinly myelinated nerve fibres and unmyelinated nerve fibres and autonomic nerve fibres.

Methods

The vibration threshold was measured over the middle of the second metacarpal on the dorsal aspect of the right hand, using a Vibrameter type III (Somedic AB, Farsta, Sweden). Vibration threshold is expressed in μm actual displacement. This method has been described in detail elsewhere [26].

Table 1. Descriptive characteristics of ORG 2766-treated and placebo-treated IDDM patients with peripheral neuropathy

	ORG 2766	Placebo
n	30	32
Age (years)	47.5 ± 12.8	47.1 ± 10.7
Sex (male/female)	15/15	24/8*
Duration (years)	24.8 ± 10.2	24.9 ± 12.4
HbA_1 (%)	9.0 ± 2.5	9.7 ± 2.3
VT (μm)	1.31 ± 1.2	1.24 ± 1.6
TTw (°C)	1.8 ± 2.7	2.2 ± 2.7
TTc (°C)	0.77 ± 1.5	1.12 ± 1.3
CAS(n/e/d/s)	7/16/6/1	9/11/9/3
MNCV – ulnar (m/s)	54.4 ± 5.7	52.5 ± 6.6
SNCV – ulnar (m/s)	48.4 ± 5.9	47.2 ± 7.9
MNCV – tibial (m/s)	37.4 ± 6.0	37.8 ± 4.7
SNCV – sural (m/s)	45.6 ± 6.4	43.6 ± 8.4
H-M interval (m/s)	30.9 ± 3.8	31.4 ± 3.7
PD (%)	45.2 ± 8.5	44.4 ± 8.7
CL (ms)	246.9 ± 42.7	245.9 ± 30.1

Data are given as mean ± SD; HbA_1, glycated haemoglobin (normal 4.0–6.5%); VT, vibration perception threshold; TTw, TTc, temperature perception threshold for warmth and cold, respectively; CAS, cardiovascular autonomic score (n, normal: 0–0.5 points; e, early involvement: 1–2 points; d, definite involvement: 2.5–3.5 points; s, severe involvement: 4–5 points); MNCV and SNCV, motor and sensory nerve conduction velocity, respectively; PD, darkness adapted pupil size; CL, pupil constriction latency

* $p < 0.01$

Temperature perception thresholds for warmth and cold were measured at the ventral side of the right wrist, using a Temperature Threshold Tester (Medelec, Old Woking, UK). This device consists of a Peltier element (thermode) and a microprocessor. The thermal threshold is expressed as the temperature change from the basal skin temperature. This method has been described in detail elsewhere [30, 31].

The five standard cardiovascular tests were performed as described by Ewing et al. [32]. (1) Blood pressure response to standing: measurement of the change in systolic blood pressure after standing from a supine position. A fall in blood pressure of less than 10 mm Hg is considered as normal, between 10 and 20 mm Hg as borderline and more than 30 mm Hg as abnormal. (2) Heart rate response to standing: calculation of the ratio from the heart rate that occurs at the 15 th and 30 th beat after standing from a supine position. A value higher than 1.03 is considered as normal, between 1.01 and 1.03 as borderline and lower than 1.0 as abnormal. (3) Beat-to-beat rate variation: measurement of change in heart rate during deep breathing. A difference of more than 14 beats per min is considered as normal, between 11 and 14 as borderline and less than 10 as abnormal. (4) Valsalva's manoeuvre: calculation of the quotient of the heart rate during and after Valsalva's manoeuvre. The patient blows into a mouthpiece, maintaining a pressure of approximately 40 mm Hg for 15 s. The ratio is calculated from the highest heart frequency during Valsalva's manoeuvre and the lowest afterwards. A ratio of more than 1.2 is considered as normal, between 1.11 and 1.2 as borderline and less than 1.11 as abnormal. (5) Blood pressure response to sustained hand grip: measurement of change in diastolic blood pressure after sustained hand grip. A rise in blood pressure of more than 16 mm Hg is considered as normal, between 11 and 15 mm Hg as borderline, and 10 or less as abnormal. Each outcome was based on the mean of three consecutive measurements. Each test was scored normal (0 points), borderline (0.5

points) and abnormal (1.0 points). The total sum of the outcome of the five tests was used for further analysis (thus varying between 0 and 5 points). Heart rate and blood pressure were measured using a Finapress tonometer (TNO, Delft, The Netherlands) [33]. The Finapress is based on servoplethysmomanometry and uses the volume-clamp technique. It measures beat-to-beat heart rate non-invasively, recording heart rate from the right index finger.

Nerve conduction velocity in the left sural nerve, the left posterior tibial motor nerve, and the right motor and sensory ulnar nerve was measured with standard surface stimulating and recording techniques (Nicolet Viking apparatus, Nicolet Biomedical Instruments, Madison, Wis., USA). Results were considered abnormal if they deviated from the normal mean ± 2.5 SD, using the data published by Oh [34]. To minimize the effect of temperature fluctuation, the limbs were warmed in water baths at 34 °C for 30 min before each measurement and were kept at this temperature with an infrared heat lamp during the examination. The Hoffmann (H)-reflex of the soleus muscle was evoked by transcutaneous bipolar electrical stimulation of the tibial nerve in the popliteal fossa [35]. Normal values for the Hoffmann-reflex latencies (H-M interval), adjusted for age and height, were derived from data published by Visser et al. [36].

Pupillary light reflexes were assessed using an infrared light reflex technique as described by Lanting et al. [37]. The pupillary system was further stimulated with a block-shaped stimulus at a fixed background and step intensity, corresponding to a retinal illuminance of 1.2 log Troland and 3.7 log Troland, respectively. The duration of the stimulus was 1.2 s. The interval between two consecutive stimuli was 5 s. The latency between the onset of the stimulus, and the start of the constriction was quantified. The latency of the constriction reaction was determined using the velocity signal, because the starting point of the constriction of the pupil is more pronounced with this derivative. The latency of the constriction to light was measured in ms using at least six artefact-free responses for both eyes. Dark-adapted pupil size was measured with the help of a slit-lamp camera. The subject was placed in front of the slit-lamp frame and was asked to concentrate on a red light-emitting diode (100 millicandela) at a distance of 5 m in order to prevent accommodation and eye movements. After focusing the pupil and iris with a three stage light intensifier system, combined with the slit-lamp camera (infrared light was used as light source), a photograph was taken with electronic flashlight. Photographs were taken after 5 min adaption to darkness. The pupil diameter percentage (horizontal pupil diameter as a percentage of iris diameter) was calculated from the photographs.

Drug

ORG 2766 is an $ACTH_{4-9}$ analogue with the chemical name L-methionyl, sulphone, L-glutamyl, L-histidyl, L-phenylalanyl, D-lysyl, L-phenylalanine. Medication was supplied to the patients in vials. Each vial contained either 3 mg of ORG 2766 or placebo in freeze-dried form. The outward appearance of the ORG 2766 and placebo vials was identical. The solvent, an ampoule containing 1 ml sterile water, was supplied with each vial. Patients injected themselves subcutaneously with 3 mg of ORG 2766 or placebo once daily (08.00 or 23.00 hours) for 12 months. Patients were instructed not to use the same injection site twice. No other treatment for peripheral neuropathy was given, except for occasional use of non-narcotic analgesic drugs. Patient compliance was checked by counting returned vials. After the 1-year trial, all patients were offered the possibility of continuing the treatment with ORG 2766 for 1 year.

Drug safety

In addition to assessing subjective complaints and to performing physical examinations, we performed the following laboratory investigations at the start of the study and after 4, 8 and 12 months: erythrocyte sedimentation rate, haemoglobin, haematocrit, total leucocyte count, platelet count, glucose, HbA_1, electrolytes, urea, creatinine, liver function tests, total protein, triglycerides, cholesterol, uric acid and urinary albumin excretion.

Statistical analysis

Baseline data are given as mean ± SD. Student's *t*-test for parametric data and Wilcoxon's rank sum test for non-parametric data were used to test for significant differences between group means at baseline. The chi-squared distribution test was used to compare the distribution of males/females over the two groups (Table 1).

For analysis of a possible treatment effect, the intention-to-treat data set was used, i.e. the data available after 4, 8 and 12 months of treatment. If the necessary requirements (normal distribution, equal variance around the regression lines and parallelism of the regression lines) were met, outcome variables were analysed by means of a one-way analysis of variance (ANCOVA) with single regression, using the baseline assessment as covariable. If the three requirements for applying an analysis of covariance were not fulfilled, a distribution-free test, the Wilcoxon's rank-sum test, was applied.

Results

The baseline characteristics of the 62 patients studied are given in Table 1. Five patients (two in the placebo-treated group and three in the ORG 2766-treated group) dropped out of the study because of problems with the daily injections of the trial medication. The other patients did not find the injections painful, and thus patients could not detect whether they were treated with ORG or placebo.The final number of patients evaluated in the placebo-treated group was 29 and in the ORG 2766-treated group, 28. Drug therapy was well tolerated. Compliance was good, as estimated by counting returned vials. Laboratory investigations did not reveal any serious side-effects.

Age and duration of diabetes were not different between the two treatment groups. The distribution of males and females was unequal, with more males being included in the placebo group. The level of metabolic control did not change in either treatment group, nor did it differ between the treatment groups at 4, 8 and 12 months.

The mean changes vs baseline at 4, 8 and 12 months in the main variables of the ORG-treated group and placebo group are given in Table 2. Vibration threshold improved significantly in the ORG group ($p = 0.05$). The other parameters of large fibre function, i.e. SNCV of ulnar and sural nerve, and MNCV of ulnar and tibial nerve, showed no difference between the two

Table 2. Mean change vs baseline in main outcome variables in ORG 2766-treated (n = 30) and placebo-treated (n = 32) IDDM patients with peripheral neuropathy at 4, 8 and 12 months

	ORG 2766			Placebo		
Month	4	8	12	4	8	12
VT (μm)	–0.17	–0.27	–0.34[a]	–0.14	–0.17	–0.20[a]
28/28	(0.40)	(0.45)	(0.24)	(0.55)	(0.49)	(0.46)
TTw	–0.31	–0.43	–0.47	–0.37	0.02	–0.69
28/28	(1.34)	(1.62)	(1.61)	(1.88)	(1.53)	(2.03)
TTc	–0.06	–0.04	–0.04	–0.25	–0.27	–0.04
28/28	(0.50)	(0.74)	(0.94)	(0.77)	(0.87)	(1.07)
CAS	0.03	0.09	0.07	0.15	0.29	0.14
28/28	(1.03)	(1.11)	(1.22)	(0.91)	(1.11)	(0.74)
MNCV – ulnar (m/s)	0.43	–0.13	0.50	–0.13	0.34	0.89
24/24	(4.79)	(4.71)	(1.26)	(0.72)	(0.96)	(0.68)
SNCV – ulnar (m/s)	–1.32	–1.81	–2.42	–1.23	–1.79	–0.50
24/24	(4.33)	(4.85)	(3.20)	(4.30)	(4.18)	(4.30)
MNCV – tibial (m/s)	0.36	0.50	0.38	–0.54	–0.35	–0.50
21/24	(3.09)	(3.16)	(3.35)	(2.93)	(2.30)	(2.98)
SNCV – sural (m/s)	–0.65	–0.88	–2.12	–0.80	–1.44	–1.08
17/12	(5.4)	(4.14)	(3.89)	(4.57)	(6.69)	(5.92)
H-M interval (m/s)	–0.21	0.01	0.18	0.02	0.07	–0.25
12/17	(2.07)	(1.54)	(1.75)	(1.57)	(1.39)	(1.60)
PD (%)	0.27	–0.52	–1.04	–0.18	1.00	0.13
24/23	(4.73)	(6.16)	(5.11)	(4.46)	(4.51)	(3.76)
CL (ms)	6.04	–2.08	12.33	–6.00	3.12	9.17
24/24	(32.86)	(29.23)	(37.90)	(32.23)	(26.29)	(23.76)

Data are given as mean change vs baseline values ± SD; VT, vibration perception threshold; TTw, TTc, temperature perception threshold for warmth and cold, respectively; CAS, cardiovascular autonomic score (n, normal: 0–0.5 points; e, early involvement: 1–2 points; d, definite involvement: 2.5–3.5 points; s, severe involvement: 4–5 points); MNCV and SNCV, motor and sensory nerve conduction velocity, respectively; PD, darkness adapted pupil size; CL, pupil constriction latency. Below each variable the number of patients in each group is given for which this value is available at 4, 8 and 12 months

* p = 0.005 for difference at 12 months

groups. Changes in the Hoffmann-reflex latency were the same in the two groups, although the H-M-interval and sural nerve SNCV could not be measured in many patients because of technical problems related to the severity of the neuropathy.

The parameters of autonomic function, i.e. cardiovascular autonomic score, dark-adapted pupil size and pupil constriction latency, did not differ significantly between the two groups.

Discussion

This study has shown that the $ACTH_{4-9}$ analogue ORG 2766 in a dosage of 3 mg s.c. every 24 h improves vibration threshold but not other neurophysiological parameters in IDDM patients with existing neuropathy. Vibration threshold is used to evaluate the function of large myelinated fibres.

The $ACTH_{4-9}$ analogue ORG 2766 belongs to the melanocortin peptide family [38]. These peptides exert a general neurotrophic effect on damaged peripheral nervous tissue. Strand and Kung [39] were the first to report an accelerated return of motor and sensory function in $ACTH_{1-39}$-treated, adrenalectomized rats after sciatic nerve crush. ORG 2766 improves nerve recovery after crush lesion and enhances peripheral sprouting in partially denervated rat muscle [40, 41]. The neuropeptide is also effective in the treatment of cis-platinin-induced neuropathy in rats [24, 25]. It was recently demonstrated in a double-blind, placebo-controlled study, that ORG 2766 is effective in preventing cis-platinin-induced neuropathy in patients with ovarian cancer [26]. Van de Zee et al. [27] demonstrated that ORG 2766 prevents the development of experimental neuropathy when given after the induction of diabetes in the streptozotocin-induced diabetic rats [27]. We have also shown that ORG 2766 is effective in the treatment of existing experimental diabetic neuropathy, both in streptozotocin- and insulin-treated BB/Wor rats [28, 29]. The pathophysiological mechanism by which ORG 2766 enhances the repair capacity of peripheral nerves after mechanical lesions in cis-platinin-induced neuropathy and in experimental diabetic neuropathy is still unclear. Experiments are in progress that specifically address the possibility that the regenerative effect of ORG 2766 in-

volves the regulation of the production of the growth-associated nervous tissue specific phosphoprotein B-50/GAP43 [42]. The dosage of ORG 2766 used in the present study was based on the dosage used in animal and human studies. The reason for the limited improvement of existing neuropathy in patients with long-standing diabetes could be caused by various factors. Only one dosage of ORG 2766 was tested, whereas the effective dosage for treatment of diabetic patients with neuropathy could be either higher or lower than this dosage. It has been shown that there is a therapeutic "window" for the effect of melanocortin analogues, including ORG 2766 [43]. These analogues exhibit an inverted U-shaped dose-response relationship and are maximally active in a dose range of 7.0–75 μg/kg s.c. when given daily or every other day. Another reason is that in the patients selected, the neuropathy may have been too far advanced for a beneficial effect to be observed. This idea is supported by the large number of patients in whom it was not possible to measure the H-M-interval and/or SNCV of the sural nerve. It has also been shown that psychophysical and neurophysiological tests have a high variability, necessitating perhaps larger patient numbers than recruited in this trial. It should be noted that the finding that only one parameter, of 13 parameters investigated, showed a significant improvement, could be due to chance. However, a second, multi-centre, dose determination trial shows similar positive results on vibration threshold (unpublished observation).

Treatment with aldose reductase inhibitors has been largely unsuccessful [16–19], or has limited effects on symptom scores, MNCV and cardiovascular autonomic function [20, 21, 44]. Recently it was shown that gangliosides, given for a month parenterally, caused a significant improvement in NCV [23]. A well-organized trial was recently published using γ-linolenic acid [45]. In a placebo-controlled trial using 111 patients γ-linolenic acid improved 13 of 16 parameters investigated. The treatment effect was partly caused by a prevention of the deterioration of neuropathy compared to the placebo-treated group, which we did not observe in our study. The failure of many interventions to improve neurophysiological parameters might be because the neuropathy has progressed too far in the patients selected, and perhaps focus should be on prevention or treatment at an earlier stage. It is imperative that metabolic control remains stable throughout the study period, because an improvement or worsening of metabolic control can neutralize or enhance a possible treatment effect in intervention trials.

We conclude that ORG 2766 improves vibration perception threshold in IDDM patients with peripheral neuropathy without changing any of the other neurophysiological parameters of nerve dysfunction. This effect on large fibres is apparently not extended to other types of fibres. In view of the reported beneficial effect of ORG 2766 in other forms of neuropathy in humans and in animals with experimental diabetic neuropathy, further testing in human diabetic neuropathy is warranted. Patient selection should focus on earlier intervention and large fibre dysfunction.

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