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Efficacy of the Neuropeptide ORG.2766 in the Prevention and Treatment of Cisplatin-induced Neurotoxicity in Rats*

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Abstract—*In rats chronic systemic treatment with cisplatin results in a sensory neuropathy as evidenced by a reduction in the sensory conduction velocity in the sciatic nerve. Concomitant administration of the neurotrophic* $ACTH_{4-9}$ *analog, ORG.*2766, *prevents the occurrence of the neuropathy. In addition, treatment with ORG.*2766 *stops further deterioration and improves recovery of an already established cisplatin-induced neuropathy. Furthermore, concomitant administration of ORG.*2766 *during a first cisplatin treatment period results in a better resistance against neurotoxicity in a second exposure period. Finally, ORG.*2766 *was shown not to hamper the anti-tumor effect of cisplatin in mice, carrying implanted tumor cells from a FMa human tumor line. These data are discussed in view of the potential clinical use of ORG.*2766 *in prevention and treatment of cisplatin-induced neuropathy.*

INTRODUCTION

CISPLATIN [*cis*-diaminedichloroplatinum(II), DDP] is an effective drug in the treatment of various types of cancer. With the inclusion of this drug in combination chemotherapy a high remission rate has been achieved in ovarian, testicular and bladder carcinoma [1]. Some reports suggest an even larger effect could be obtained if the drug was administered in higher dosages [2]. A clear dose–response relationship has been shown in ovarian cancer [3]. It is not likely that cisplatin will be totally replaced by its analogs, such as carboplatin, in the near future [4]. Unfortunately, a dose-dependent and predominantly sensory peripheral neuropathy limits the repeated use of high dosages [5]. In patients the onset of the neuropathy will be usually expressed by paresthesia and numb feelings in hands and feet, becoming apparent at cumulative doses between 300 and 600 mg/m^2 [6–9]. These symptoms worsen progressively and may eventually lead to a debilitating ataxia, rendering the patient wheelchair-dependent [2]. Though most of the time the neuropathy tends to be reversible upon cessation of therapy, recovery is slow and often incomplete [2, 10]. Decreased vibration sense has been shown to precede the clinical manifestation of this neuropathy [11]. This as well as histological findings favor the explanation that the thickest afferent nerve fibers are first affected in this drug-related side-effect [10, 12].

Recently, a model has been developed to study cisplatin neurotoxicity in rats [13]. Using this model it could be shown that concomitant treatment with the neurotrophic peptide ORG.2766, an $ACTH_{4-9}$ analog devoid of the adrenocorticotrophic effects of the parental peptide, prevents cisplatin neuropathy in rats [14]. In an immunocytoma model no adverse effects of this peptide on the anti-tumor activity of cisplatin were observed [14].

In this paper we present the results of three separate studies. In the first experiment we investigated whether ORG.2766 can be of benefit when a neuropathy already exists. The second experiment was performed to study whether previous administration of ORG.2766 results in a more favorable starting condition for a second treatment period with cisplatin. Finally, the possible effect of ORG.2766 on the anti-tumor activity of cisplatin was investigated in a human tumor line of gynecological origin.

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MATERIALS AND METHODS

Animals

Female Wistar rats of an inbred strain (TNO, Zeist, The Netherlands) were used in all experiments on cisplatin neurotoxicity. Their weight varied between 190 and 210 g (age 12–13 weeks). The animals were housed in Macrolon cages (RUCO, The Netherlands), four in a cage, on sawdust with food and water available *ad libitum*. A dark–light cycle of 12 h was maintained with lights on from 7.30 am till 7.30 pm.

The possible interaction between ORG.2766 and cisplatin was studied in female tumor-bearing nude mice (TNO, Zeist, The Netherlands), 8–10 weeks of age.

Drug administration

Cisplatin was administered by intraperitoneal injection to rats, using a solution of 25 mg cisplatin in 50 ml solvent (Platinol, Bristol-Myers, Weesp, The Netherlands) which was diluted with saline to a final concentration of 0.04 mg/ml. The injections (1 mg/kg body wt) were given twice a week (on Tuesday and Friday) and were preceded (20 min) by a subcutaneous injection of furosemide (12.5 mg/kg body wt; Lasix, Hoechst, F.R.G.) in order to prevent nephropathy.

The nude mice received intravenous injections of cisplatin (5.0 mg/kg body wt) on day 0 and day 7: ORG.2766 [H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH; Organon Int. BV, Oss, The Netherlands], a degradation resistent $ACTH_{4-9}$ analog, was dissolved in 0.9% NaCl to a concentration of 20 μg/ml and was given subcutaneously (10 μg/rat), four times a week (on Tuesday, Thursday, Friday and Sunday). The nude mice received ORG.2766 (1.5 μg) 10 min prior to and 2 days after the administration of cisplatin.

Tumor induction

Human tumor line FMa (poorly differentiated mucinous adenocarcinoma of gynecological origin) was transplanted subcutaneously in tissue fragments 2–3 mm in diameter in both flanks of 8–10 week old animals. At the start of treatment tumors had a mean volume of 50–150 mm^3. Tumors were measured weekly in three dimensions by the same observer with a slide caliper. The ratio of mean relative volumes of treated tumors over that of control tumors multiplied by 100 (T/C%) was calculated to measure the efficacy of the drugs involved as described in detail by Boven *et al.* [15].

Electrophysiology

Electrophysiological measurements were performed under general anesthesia using Hypnorm (Duphar, Weesp, The Netherlands) containing fluanisone (10 mg/ml) and phentanyl citrate (20 mg/ml), at a dose of 0.8 ml/kg body wt. Motor nerve conduction velocity (MNCV) and H-reflex related sensory nerve conduction velocity (HSNCV) were measured using the method described by De Koning *et al.* [13]. The H-reflex is a long latency reflex which occurs in response to stimulation of the Ia-afferent that monosynaptically excites α-motoneurons in the spinal cord (Fig. 1). In short, the MNCV and HSNCV were measured as follows: the sciatic and tibial nerve were stimulated at the sciatic notch and ankle and the M-response and H-reflex were recorded from the small muscles of the foot by means of surface electrodes.

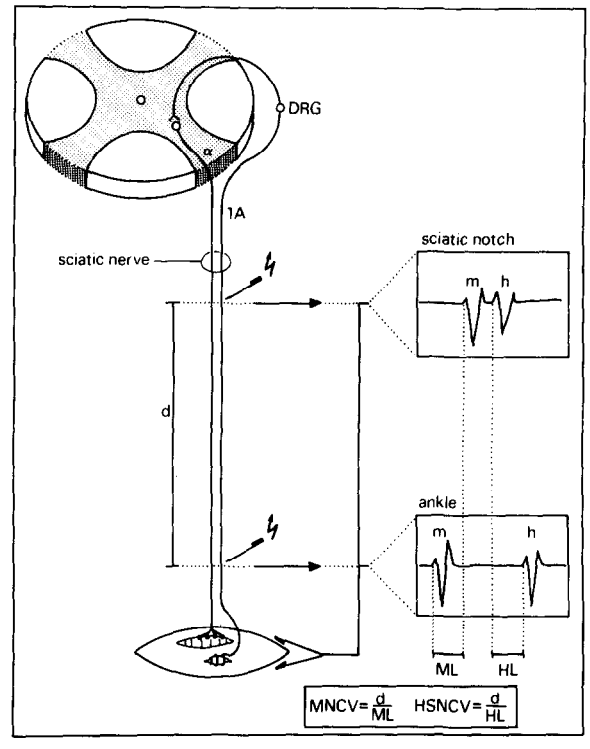

Fig. 1. Illustration of the electrophysiological method used for measuring the motor nerve conduction velocity (MNCV) and the H-reflex-related sensory nerve conduction velocity (HSNCV). The M-response and the H-reflex are recorded from the plantar muscles of the foot using surface electrodes after stimulation of the sciatic nerve at the sciatic notch (close to the hip) and the tibial nerve at the ankle. (DRG = dorsal root ganglion, α = α-motor neuron). 1A = 1A sensory afferent fiber, d = distance between stimulation points.)

MNCV and HSNCV were calculated by dividing the distance between the two stimulation points by the difference in recorded M- and H-latencies at both points.

Experimental design

In order to study the beneficial effect of ORG.2766 on cisplatin neuropathy, 20 rats received cisplatin for 11 weeks up to a cumulative dose of 22 mg/kg body wt. Subsequently, they were randomly divided in two groups of 10 rats each with continued cisplatin treatment in both groups. One of the groups was given ORG.2766 as sup-

plementary treatment, whereas the other received concurrent saline injections. The combined treatment regimen was applied for another 6 weeks. A group of five age-matched animals without any treatment served as a control.

To investigate possible long-term beneficial effects of ORG.2766, two groups of 12 rats were treated with cisplatin during 12 weeks up to a cumulative dose of 24 mg/kg. One group received concomitant injections of ORG.2766, while the other group was given concomitant saline injections. During this period the animals were subjected to repeated electrophysiological examinations. Subsequently, all animals were allowed to recover for 15 weeks without any treatment. Following this recovery interval the rats were again treated with cisplatin for a period of 7.5 weeks. This time no concurrent treatment with ORG.2766 or saline was given.

Finally, in the third experiment 18 mice bearing subcutaneous xenografts of human tumor line FMa were randomized in three groups of six animals each. The control group received no treatment. The other two groups were treated with cisplatin and received ORG.2766 or saline as concurrent medication. Repeated tumor measurements were carried out for a period of 8 weeks.

All experiments were performed in a blind fashion. The treatment code was broken only after data analysis had been performed.

Statistics

The results were analyzed using an analysis of variance with repeated measures followed by supplemental *t*-tests (*P* values are shown in the figures).

RESULTS

Two animals died in the first experiment, one in each group. After a cumulative dose of cisplatin of 22 mg/kg body wt the HSNCV decreased in both treatment groups to 67% as compared to control animals (Fig. 2). A recovery of the HSNCV (74%) could be observed in animals receiving ORG.2766 as co-treatment during the final 6 weeks of the experiment. In rats that received saline as supplementary treatment, the HSNCV further decreased to 63%. The difference between ORG.2766 and saline-treated groups was statistically significant ($F = 74$, $P < 0.001$).

In the second experiment five rats died in the cisplatin/saline-treated group and two in the cisplatin/ORG.2766-treated group. During the entire experiments no effects of cisplatin were seen on the MNCV in either of the groups. At a cumulative dose of cisplatin of 13 mg/kg the HSNCV in cisplatin/saline-treated rats started to decrease (Fig. 3), reaching a value of 75% at a cumulative dose of cisplatin of 24 mg/kg. No such decrease was

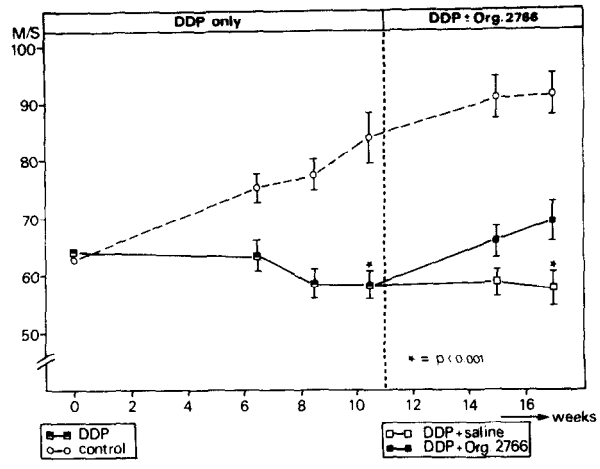

Fig. 2. Effect of treatment with ORG.2766 starting at a cumulative dose of cisplatin (DDP) of 22 mg/kg. *On the vertical axis is depicted the H-reflex related sensory nerve conduction velocity in* m/s. *The difference between the ORG.2766-treated group (black squares) and the saline-treated group (open squares) is statistically significant* ($P < 0.001$).

observed in cisplatin/ORG.2766-treated animals ($F = 47$, $P < 0.001$). At the end of the recovery period the HSNCV had returned to control levels in all inflicted animals. When exposed again to cisplatin treatment, all animals developed signs of a sensory neuropathy on electrophysiological examination. However, the decrease in HSNCV was much faster and to a much lower level in rats formerly treated with cisplatin/saline than in those previously treated with ORG.2766 as co-treatment. At the end of this second cisplatin exposure period the HSNCV had decreased to 70% and 86%, respectively ($F = 9.49$, $P < 0.01$).

Results of the third experiment are shown in Fig. 4. Relative tumor volumes in both cisplatin/saline and cisplatin/ORG.2766-treated mice decreased to levels of 25% and 32% on day 26, respectively. An exponential growth of the tumor could be observed in untreated mice ($P < 0.001$). The difference in tumor growth between the two treatment groups was not significant.

DISCUSSION

These experiments confirm that ORG.2766 protects against cisplatin-induced neuropathy [14] and in addition show that the drug is able to improve recovery of sensory nerve conduction in rats already suffering from a cisplatin neuropathy. The beneficial effects of ORG.2766, when administered during a first cisplatin treatment period, could also be shown during a subsequent second course of cisplatin exposure. Finally, ORG.2766 does not hamper the anti-tumor activity of cisplatin in nude mice bearing implanted xenografts of the human FMa tumor line.

Normally in rats motor and sensory nerve conduction velocity steadily increase during the first 6 months of life [14]. In the present rat model a

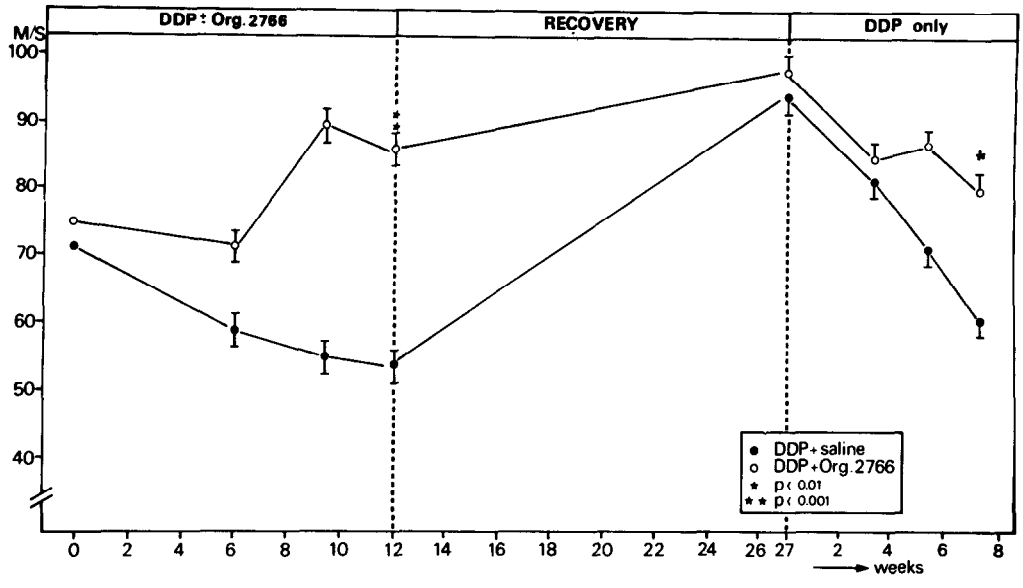

Fig. 3. Effect of ORG.2766 treatment administered during a first course of cisplatin (DDP) on development of a neuropathy during a following exposure period. On the vertical axis is depicted the H-reflex-related sensory nerve conduction velocity in m/s *(difference between groups at* 12 *weeks* $P < 0.001$; *at* 34 *weeks* $P < 0.01$).

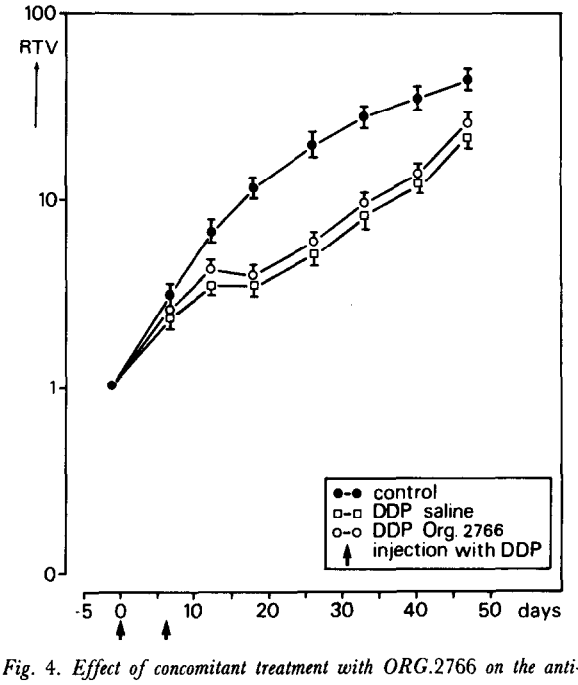

Fig. 4. Effect of concomitant treatment with ORG.2766 on the anti-tumor activity of cisplatin (DDP) in mice bearing xenograft tissue of a human FMa tumor line. The difference between cisplatin-treated groups is not significant (RTV = relative tumor volume).

slowing in the HSNCV is observed as a consequence of cisplatin treatment. Using this model a protective effect of the neurotrophic peptide ORG.2766 on cisplatin neuro-toxicity was recently shown in rats, when given concomitantly with cisplatin from the start of the treatment [14]. The beneficial effects of neurotrophic peptides derived from ACTH/α-MSH on regeneration of peripheral nerve following a crush lesion have been extensively documented [16, 17]. The reported enhancement of recovery is caused by an influence on the initial sprouting response. In general, the neurotrophic properties of melanocortins become only apparent when an inadequate state of nervous system function exists, e.g. during development of following a disturbance of the balance between degeneration and regeneration such as traumatic injury or treatment with a neurotoxic agent (cisplatin) [17].

Cisplatin-induced neuropathy is predominantly a sensory peripheral neuropathy, in which the modalities conveyed through larger, thickly myelinated fibers (like vibratory sense and fine touch perception) are primarily affected [8, 10]. A number of investigators reported changes in sensory conduction in patients treated with cisplatin. Both a decrease of amplitude and of conduction velocity as well as a prolonged sensory latency were noted [10–12]. No clear mechanism, through which cisplatin interacts with nervous tissue, has yet been identified. Information deduced from sural biopsies indicates secondary myelin breakdown occurs as a possible consequence of earlier axonal changes, resulting in a dying-back-like neuropathy. The dorsal root ganglion has been proposed as the primary target of cisplatin, accounting for the sensory nature of the neuropathy [10, 12]. The effects of prolonged cisplatin treatment on myelin thickness, axon numbers and dorsal root ganglia need further investigation.

The results of the first experiment in the present study prove that ORG.2766 can not only prevent the occurrence of a cisplatin polyneuropathy, but can also exert a beneficial influence in rats in the presence of a cisplatin neuropathy. In rats starting treatment with ORG.2766 at a cumulative dose of cisplatin of 22 mg/kg body wt the HSNCV did

not decrease further but even increased while it remained depressed in saline-treated rats. These results suggest that treatment with ORG.2766 may be considered in patients already disabled by a manifest cisplatin neuropathy.

Treatment with ORG.2766 during a first course with cisplatin in part protects nerve cells against the neurotoxic effects of a second course of cisplatin treatment. In the second experiment all rats previously treated with cisplatin developed signs of neuropathy in a next exposure period after a recovery interval in which HSNCV values had returned to normal in all animals. However, the onset of the neuropathy was much slower and the decrease in HSNCV less profound in animals previously co-treated with ORG.2766 when compared to animals, previously not treated with this neuropeptide.

The results of the third experiment, in which cisplatin and ORG.2766 were administered to mice bearing human tumor xenografts of gynecological origin show that ORG.2766 does not diminish the anti-tumor effect of cisplatin in this particular model. These findings are in agreement with results of a study in which ORG.2766 did not influence the cisplatin efficacy in an immunocytoma model in rats [14].

The positive results of ORG.2766 in cisplatin-treated rats and the absence of signs of an interaction with the anti-tumor activity of cisplatin warrant a clinical trial of ORG.2766 in patients treated with cisplatin-based drug regimens. No side-effects of ORG.2766 have been reported in human studies [18]. Recently, a multi-centered, placebo-controlled, dose finding study using two dose levels of ORG.2766 has been initiated in cisplatin-treated ovarian cancer patients by the Netherlands Joint Study Group for Ovarian Cancer. Successful protection from neurotoxicity would not only be highly beneficial to patients receiving cisplatin-based chemotherapy, but would also enable cisplatin regimes with higher doses in the future. In addition, patients already suffering from cisplatin neuropathy might also benefit from ORG.2766 treatment. Protection from neurotoxicity is an important aspect for further studies with ORG.2766 in order to achieve a better therapeutic index of the widely used, effective anti-tumor agent cisplatin.

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