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ACTH/MSH LIKE PEPTIDES IN THE TREATMENT OF CISPLATIN NEUROPATHY

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Summary—The neurological toxicity seen in patients treated with cisplatin in most cases concerns ototoxicity and peripheral neuropathy. Thus far, the pathogenesis of cisplatin neuropathy remains obscure. Yet the fact that cisplatin affects mainly the sensory peripheral nerve fibers points towards an involvement of the dorsal root ganglia. In a rat model of cisplatin neuropathy, following a cumulative dose of approx. 12 mg/kg cisplatin the sensory nerve conduction velocity began to slow as compared to age-matched controls. Peptides derived from ACTH and MSH are known to exert neurotrophic effects. *In vivo* they facilitate postlesion repair mechanisms in the peripheral nervous system by enhancing the early sprouting response of the damaged nerve. Surprisingly, chronic treatment with a synthetic $ACTH_{4-9}$ analog not only prevented cisplatin neurotoxicity following a low or high dose regimen, but also counteracted already existing cisplatin-induced neurotoxicity. Stimulated by these findings a randomized, double blind, placebo-controlled study was performed to assess the efficacy of the peptide in the prevention of cisplatin neuropathy in women suffering from ovarian cancer. The threshold of vibration perception (VPT) was used as the principal measure of neurotoxicity. Following 6 cycles of chemotherapy the VPT had increased more than 8-fold in women receiving placebo as co-medication. Whereas the VPT in women receiving 1 mg/m^2 body surface $ACTH_{4-9}$ analog before and after each cisplatin cycle only increased <2-fold. No side effects of the peptide treatment were observed and the clinical response to the chemotherapy was similar in all treatment groups. Collectively these preclinical and clinical data suggest that treatment based on non-endocrine fragments of ACTH/MSH may be a therapeutic option in the treatment of cisplatin neuropathy.

INTRODUCTION

Although the regenerative capacity of the peripheral nervous system is limited—damaged neurons cannot be replaced—it is not nihil; when damage is restricted to the neuronal processes (dendrites and axons) regeneration and reinnervation are possible.

In the last decade, stimulation of nerve regeneration by pharmacological methods has been extensively explored. Nerve repair appears to be facilitated by numerous factors, both humoral and structural [1]. The first studies on the promotion of peripheral nerve regeneration were performed in experimental models of mechanical nerve damage (nerve crush or transsection). To date, the investigation of putative neurotrophic factors takes other aspects of peripheral nerve disease into account (e.g. neuropathies induced by toxins, diabetes mellitus, demyelinating disease), sometimes with considerable success.

Cisplatin is an oncolytic drug that is very efficient against a number of tumours, most notably those of the ovary and testis. Its use is however severely restricted by the induction of a peripheral neuropathy that leads to a very disabling sensory ataxia. As such, methods to overcome this toxicity are clinically very important.

In this paper we present the rationale for and the first results of the use of melanocortins (ACTH/MSH like peptides) in the treatment of cisplatin-induced neuropathy.

NEUROTROPHIC PEPTIDES

Since pioneering studies by De Wied [2] suggested that pituitary peptides may directly modulate nervous system function and behaviour, a tremendous amount of information

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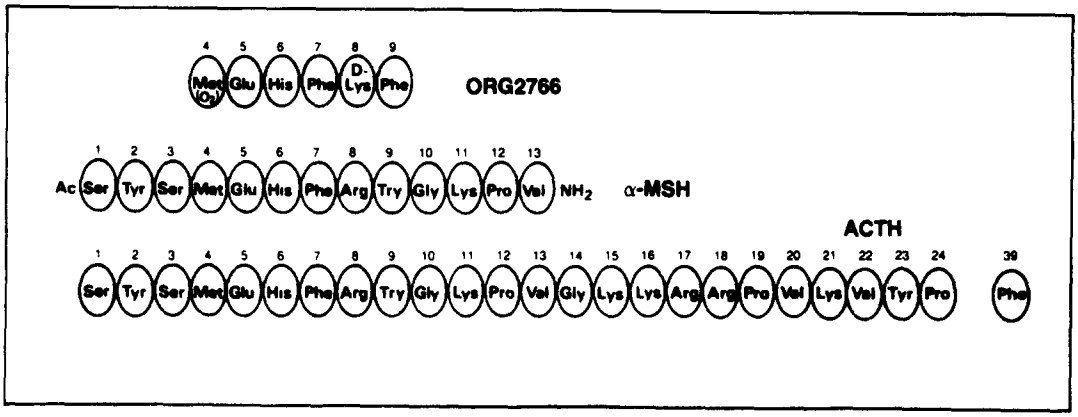

Fig. 1. Structures of some peptides of the melanocortin family.

on the actions of these peptides and their synthetic fragments and analogs on the nervous system has been compiled.

The melanocortins, peptides related to the pituitary hormones ACTH and MSH, as hormones mediate a variety of adaptive responses, and, in considering whether neural tissue is also a target for these peptides, various investigators have studied the possibility that melanocortins may exert effects on the nervous system analogous to their known trophic effects on their classical target organs [3]. Initially peptides with full endocrine activity were used; the rationale behind the treatment was that corticosteroids released by ACTH would reduce the formation of scar tissue, which was believed to limit proper neural tissue regeneration. However, corticosteroids are nowadays often known to exert catabolic rather than anabolic effects and this may have disturbed the final outcome in previous studies [4]; indeed studies on the peripheral nervous system have shown that the corticotrophic activity of melanocortins is not involved in the trophic response of neural tissue. The first to describe this fact were Strand and Kung in 1980 [5]: they showed that $ACTH_{1-39}$,—the full amino-acid sequence of ACTH—accelerated recovery from peripheral nerve lesion in adrenalectomized rats, as compared with saline treated rats. Clearly, in the adrenalectomized rat peptide treatment cannot stimulate the secretion of corticosteroids [5].

Using a foot reflex withdrawal and a free-walking pattern analysis test to monitor return of nerve function after a sciatic nerve crush lesion, we have found that neurotrophic fragments and analogs of melanocortins, including the $ACTH_{4-9}$ analog ORG2766 (Fig. 1), exhibit an inverted U-shaped dose–response relationship: the peptides are maximally active in a dose range of 7–75 μg/kg when administered daily or every other day s.c. [6, 7]. Peptide treatment, furthermore, must begin within a short period of induction of the lesion to be efficient [8], but its beneficial effects on histological and electrophysiological parameters are still apparent after several months [9, 10]. The precise location of the amino-acid sequence that confers neurotrophic activity on the peptides is uncertain. It is clear however that the critical information for stimulating neurite outgrowth is contained in the amino acid-sequence between positions 4 and 10; the peptide ORG2766 ($ACTH_{4-9}$ analog) is frequently used in studies of these effects [11].

Histological and functional studies strongly support the notion that the melanocortins do not enhance the rate of outgrowth but rather increase the number of newly formed nerve sprouts at the site of the lesion [4]. It has been suggested that the peptides mimic or amplify an endogenous signal that operates early in the regenerative response of the damaged neuron [4]. If this is indeed the case, it may be speculated that the melanocortins will have a broader therapeutic role than enhancing post-lesion repair alone: it may be possible to exploit the regenerative repertoire of neurons compromised by a variety of toxins or metabolic dearrangements to counteract the harmful effects of these conditions.

CISPLATIN NEUROPATHY

In the last decade an increasing number of papers has been published on the neurotoxicity of anticancer agents. With the improvement of supportive care the administration of higher

cumulative dosages is now a reality and as a consequence neurotoxic effects are encountered more frequently.

To date, cisplatin is rated as one of the most effective oncolytics available: however, it gives rise to a number of serious side-effects. Among the main toxicities induced by cisplatin are those of short-term nature such as nausea and vomiting and the more threatening, long lasting dangers as oto-, nephro- and neurotoxicities. For a number of years, renal toxicity was the dose-limiting side-effect, but since this toxicity can be reduced by such procedures as forced hydration, diuretics and a slow rate of infusion of the drug, more aggressive dosing schemes can be employed and presently neurotoxicity has become the major and dose-limiting side-effect.

Neurological disease occuring in patients treated with cisplatin is limited almost completely to the peripheral nervous system. Less frequent signs of neurotoxicity described in the literature include Lhermitte's sign, retrobulbar neuritis, encephalopathies and an autonomic neuropathy. These signs are however quite rare. The peripheral neuropathy is of a sensory nature with preferential decrease of such thick myelinated fiber qualities as vibration and position sense; there seems to be no motor involvement [12, 13].

The first symptoms are those of a bilateral sensory neuropathy with numbness and tingling, often in a stocking and glove distribution. As the neuropathy progresses position sense becomes impaired. The sensory ataxia that develops ruins fine motor skills (writing, clothing) and walking. These symptoms are often accompanied by uncomfortable and painful paraestesias. Muscle strength remains essentially the same.

Before any symptoms become clinically evident, an increase in vibration perception threshold may be measured [14]. Quantitative measurement of this threshold is a relatively simple, accurate and reliable technique to monitor this neuropathy, and as such can also be used for the evaluation of proposed neuroprotective therapies.

The neuropathy induced by cisplatin becomes more severe with increasing cumulative doses. The Netherlands Joint Study Group for Ovarian Cancer reported the overall incidence of neurotoxicity in any grade of severity as 47% [15]. Of this, about equal percentages of patients suffered from a mild or a moderate degree of neurotoxicity. The mean cumulative cisplatin dose after which neuropathic symptoms can be found was between 500–600 mg/m^2.

Cisplatin neuropathy is more frequently observed in high-dose treatment regimes, due to the larger cumulative dose given to these patients and not to increased toxicity of individual infusions containing a high dose of cisplatin. This form of high-dose cisplatin therapy (200 mg/m^2/course as compared to 75–100 mg/m^2/course in normal treatment regimens) is currently extensively studied for the treatment of various solid tumors. By maintaining acceptable nephrotoxicity levels, dose–response relationships have shown increased efficacy of cisplatin therapy. However, severe neurotoxicity and myelosuppression, prevented further dose increases [16]. The neuropathy, however, may continue to develop for some months after discontinuation of cisplatin treatment, or become clinically evident first then.

NEUROTROPHIC PEPTIDES IN CISPLATIN-INDUCED NEUROPATHY

Animal studies

In order to test the notion that melanocortins might stimulate nervous recovery from damage induced by toxins, De Koning [17] developed a rat model for cisplatin neuropathy: by biweekly injections of cisplatin (1/mg/kg/injection i.p.) a sensory neuropathy, as evidenced by a decrease in the H-reflex related sensory nerve conduction velocity, was induced. This decrease became significant from 6 weeks (12 mg cisplatin/kg cumulative) onwards. This conduction velocity is dependent on the thickest myelinated sensory nerve fibers. This correlates rather well with the human clinic in which it is also the thickest myelinated sensory nerve fibers that degenerate.

Using this model de Koning was able to show that concomitant administration of the neurotrophic peptide ORG2766 in a dose of 75 μg/kg every other day could protect from the cisplatin-induced decrease in nerve conduction velocity observed in rats co-treated with saline [17]. Subsequent reports on the protecting effects of ORG2766 in this animal model followed [18, 19].

As one of the rationales of peptide treatment in this particular neuropathy is to increase cisplatin dose-intensity we have recently performed experiments employing a 2-fold higher cisplatin dose per time-unit, in combination

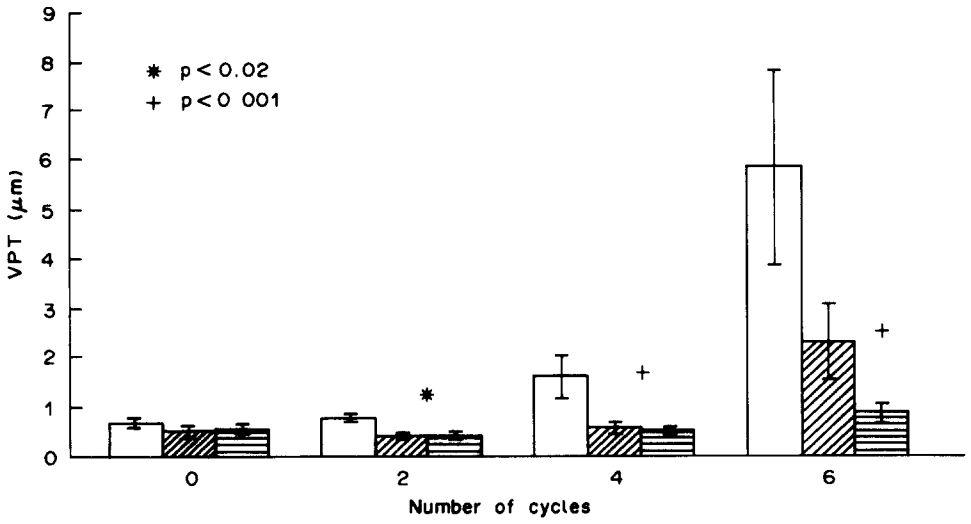

Fig. 2. Vibration perception thresholds (VPT) in μm (±SEM) as measured before chemotherapy and after 2, 4 and 6 treatment cycles (75 mg cisplatin/m²/treatment cycle). Open bars: placebo group; diagonal hatched bars: low (0.25 mg) ORG2766; horizontal hatched bars: high (1 mg) ORG2766.

with ORG2766. Our results indicate that the peptide prevents the neuropathy even in a high dose-intensity cisplatin treatment [20].

As in our animal model for cisplatin neuropathy we employ young adult rats, it could be argued that the cisplatin neurotoxicity observed is related to a slowing of the maturation of the peripheral nerve and thus does not closely resemble the clinical situation. However, in a recent study with fullgrown adult rats (7–10 months of age) we observed a cisplatin-induced slowing of the sensory nerve conduction velocity which could be counteracted by concomitant peptide treatment [Hamers, in preparation].

Clinical studies

Based on the experimental evidence as outlined above, a randomized, double-blind, placebo-controlled study was initiated to assess the efficacy of ORG2766 in the prevention of cisplatin neurotoxicity in women with ovarian cancer. The peptide was administered s.c. in doses of respectively 0.25 mg (low dose) and 1.0 mg (high dose) per m² of body–surface area before and after each cisplatin application in a combination chemotherapy with cisplatin and cyclophosphamide (75 and 750 mg/m² every 3 weeks). The principal measure of neurotoxicity was the vibration perception threshold. After four cycles of chemotherapy, the mean (SE) threshold value for vibration perception in the placebo group increased from 0.67 (0.12) to 1.61 (0.43) microns of skin displacement. In the high-dose treatment group, there was no increase in the threshold value after four cycles [from 0.54 (0.12) to 0.50 (0.06) microns]. After six cycles of chemotherapy, the threshold value was 5.87 (1.97) microns in the placebo group (a >8-fold increase from base line), as compared with 0.88 (0.17) microns (a <2-fold increase) in the high-dose group (Fig. 2). Moreover, in the high-dose group, fewer neurological signs and symptoms were recorded than in the placebo group. With the lower dose of the peptide, the effects were less prominent. No side-effects were seen after treatment with ORG2766. Especially the rates of clinical response to chemotherapy were the same in all three groups [21]. These results suggest that ORG2766 can prevent or attenuate cisplatin neuropathy.

Based on this study further clinical trials are presently being performed in Europe, the United States, Japan and Australia in patients with both testicular and ovarian cancer.

CONCLUSIONS

During the last decade or so the neurotrophic properties of melanocortins have been extensively studied. Based on the effectiveness of peptide treatment in facilitating postlesion plasticity in various animal models of peripheral nerve disease we have suggested that the peptides of the ACTH/MSH family enhance the repair capacity of peripheral nerves in general.

The clinical use of neurotrophic compounds in the treatment of mechanical damage, the model in which they were initially investigated

is not to be expected in the near future: for the variability of this lesion and its repair technique employed is such that standardized clinical trials to test the efficacy of neurotrophic factors in promoting faster and/or better reinnervation of the target organs are difficult to perform.

In contrast, the onset and development of an iatrogenic nerve disorder such as a cisplatin-induced neuropathy can be monitored much more readily. Indeed in an animal model of cisplatin neurotoxicity co-treatment with the neurotrophic peptide resulted in suppression of this neurotoxicity. In the first clinical study in which ovarian cancer patients received cisplatin chemotherapy co-treatment with the peptide greatly ameliorated the clinical signs and symptoms of the cisplatin neuropathy.

Further pre- and clinical work is in progress to substantiate these initial findings.

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