

## RESEARCH NOTE

### Org.2766 Protects from Cisplatin-Induced Neurotoxicity in Rats

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One of the side-effects of the cytotoxic drug, cisplatin, is its neurotoxicity. In rats this neurotoxicity can be measured as a slowing of the H-reflex-related sensory nerve conduction velocity. Concurrent treatment with Org.2766 (an ACTH<sub>4-9</sub> analog) prevents this neurotoxic side effect while leaving the antitumor activity of cisplatin unaffected. © 1987 Academic Press, Inc.

The cytotoxic drug, cisplatin [*cis*-diaminedichloroplatinum (II)], has shown particular efficacy against bladder, testis, and ovarian cancers. However, unwanted side effects, particularly neurotoxicity, set limits to its therapeutic use. The neurotoxicity manifests itself as a primarily sensory neuropathy although retrobulbar neuritis and neurosensory hearing loss have also been reported (10). The first objective sign of the neuropathy is a diminution of vibratory sensibility probably resulting from an impairment of the widest afferent nerve fibers, followed by a prolongation of the distal sensory latency

Abbreviations: BW—body weight; HSNCV, MNCV—H-reflex-related sensory, motor nerve conduction velocity.

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and slowing of the sensory conduction velocity (6). Signs of involvement of motor nerve fibers are seldom observed.

In recent years, numerous studies revealed neurotrophic effects of melanocortins [peptides derived from adrenocorticotrophic (ACTH) and melanocyte-stimulating (MSH) hormones] on central and peripheral nervous tissue in situations in which neural plasticity is most expressed: regeneration and development of the nervous system (3, 8). The beneficial effects were observed with ACTH/MSH derivatives and analogs that lack the classical endocrine and metabolic influences of the parent molecules. Extensive studies were carried out on the neurotrophic properties of the degradation-resistant ACTH<sub>4-9</sub> analog Org.2766. Acute and (sub)chronic (10 days to 12 months) safety studies in rats, dogs, and humans with oral, subcutaneous, and intravenous doses ranging between 0.1 and 100 mg/kg body weight did not reveal any serious toxic effects on blood biochemistry, hematology, hormones, or liver and kidney function (2, 5). After systemic administration in the rat, Org.2766 enhances and improves recovery of damaged peripheral nerves as judged by functional, electrophysiological, and histological studies (1, 8).

We recently devised an animal model to assess the neurotoxic side effects of cisplatin treatment. Female rats (body weight about 200 g, age 13 weeks) of an inbred Wistar strain (TNO, Zeist, NL) were treated with cisplatin (Platinol, Bristol-Myers, Spain) 1 mg/kg body weight (BW), i.p., twice a week (on Tuesday and Friday) to a cumulative dosage of 19 mg/kg BW. To prevent nephrotoxic side effects, all rats received furosemide (Lasix) 12.5 mg/kg BW, s.c., 20 min prior to each cisplatin administration. The sciatic and tibial nerve were stimulated at the sciatic notch and ankle, respectively, by means of monopolar needle electrodes. Upon stimulation of these mixed peripheral nerves, two concomitant responses can be recorded from the small muscles of the foot through surface electrodes. The first response has a short latency, is called the M-response, and occurs in response to direct stimulation of the  $\alpha$ -motor fibers. The second response which is recorded simultaneously with the M-response is called the H-response. The H-reflex occurs in response to stimulation of the afferent Ia-fibers which monosynaptically excite  $\alpha$ -motor neurons in the spinal cord. From the latencies of these responses and the distance between the two stimulation points, the motor (MNCV) and H-reflex-related sensory nerve conduction velocity (HSNCV) can be calculated (7). The electrophysiological investigations were carried out at the start of the experiment and at various times throughout the investigation period. In cisplatin-treated rats, we consider a slowing of either of these velocities or the occurrence of denervation potentials measured with a concentric needle electrode indicative of a neuropathy. This method was used to study the possible efficacy of Org.2766 in preventing the neurotoxic side effects of cisplatin. Furthermore, we investigated whether or not Org.2766 hampers the antitumor activity of cisplatin.

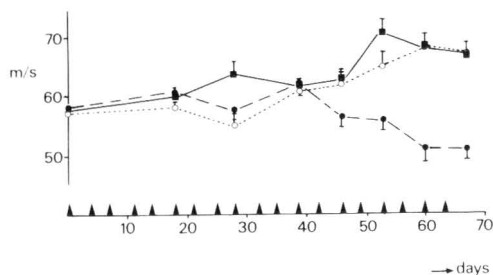


FIG. 1. Alterations in the H-reflex-related sensory nerve conduction velocity during cisplatin intoxication. Values  $\pm$  SE. ■—Mean values of control animals ( $N = 9$ ). ○—Mean values of animals treated concurrently with cisplatin and Org.2766 ( $N = 11$ ). ●—Mean values of animals treated concurrently with cisplatin and saline ( $N = 8$ ). ▲—Indicates administration of cisplatin 1 mg/kg body weight.

*Effects of Org.2766 on Cisplatin Neurotoxicity.* Twelve cisplatin-treated rats received 10  $\mu$ g Org.2766 (Organon Int. BV, Oss, NL) subcutaneously four times per week (on Sunday, Tuesday, Thursday, and Friday), and another 12 cisplatin-treated rats received saline injections. Ten untreated female rats served as controls. The rats were injected in such a way that the experimenter was unaware of the treatment the rats received. The treatment code was broken after data analysis. After 47 days (cumulative dose of cisplatin 13 mg/kg bw), the HSNCV in the cisplatin/saline-treated rats began to slow and at 67 days (cumulative dosage of cisplatin 19 mg/kg bw) was 76% of that of the control animals ( $F = 47$ ,  $P < 0.001$ ). In contrast, in the group of cisplatin/Org.2766-treated rats no slowing of the HSNCV occurred (Fig. 1). In none of the groups were changes in the MNCV or denervation potentials observed. Data from animals that died before the end of the experiment were not included in the statistical analysis. Four animals died in the cisplatin/saline-treated group of rats, whereas only one animal died in the cisplatin/Org.2766-treated group and also one animal in the control group. The mortality observed might have been caused by repeated anesthesia of rats that were debilitated by cisplatin.

Therefore, in a second experiment, similar to experiment 1 rats were anesthetized only four times in order to conduct an electrophysiological examination, i.e., at commencement of the experiment and at a cumulative dosage of cisplatin of 13, 16, and 19 mg/kg BW, respectively. In this experiment only one animal died in the cisplatin/saline-treated group and none in the cisplatin/Org.2766-treated group. Again, after 47 days the HSNCV only slowed in the cisplatin/saline-treated group and at 67 days (cumulative dosage of cisplatin 19 mg/kg bw) it was 62% of that of the untreated control animals ( $F = 54$ ,  $P < 0.001$ ).

TABLE 1

Tumor Diameter on Successive Days following Commencement of Cisplatin Therapy<sup>a</sup>

	Day						Tumor reduction
	0	3	7	10	14	17	
No treatment (6)	6.8 ± 4.3	15.6 ± 4.7	26.6 ± 2.7	31.9 ± 2.4	— <sup>b</sup>	—	—
Cisplatin (6)	8.9 ± 3.3	11.5 ± 2.2	6.3 ± 2.7	4.1 ± 3.6	2.8 ± 3.4	1.5 ± 1.6	83%
Cisplatin + saline (10)	9.5 ± 2.6	11.3 ± 1.4	7.2 ± 2.8	3.2 ± 3.2	1.1 ± 2.6	0.7 ± 2.2	93%
Cisplatin + Org.2766 (10)	9.3 ± 2.6	11.7 ± 1.4	4.7 ± 2.2	1.7 ± 2.6	0.3 ± 0.9	0.8 ± 2.3	91%

<sup>a</sup> Values are  $\bar{x} \pm 1$  SD, tumor diameters in millimeters; number of animals in parentheses.<sup>b</sup> No measures are indicated in the untreated group of rats from day 10 onward as these animals had died before day 14.

*Effect of Org.2766 on Antitumor Activity of Cisplatin.* In 32 female Lou/M Wsl inbred rats (RIVM, Bilthoven, NL)  $2.10^4$  IgM-immunocytoma cells in 0.5 ml plain Roswell Park Memorial Institute Medium 1640 (GIBCO, Europe BV, Hoofddorp, NL) were inoculated s.c. on the left flank. The growth of the tumor was measured twice a week with vernier calipers and expressed as the mean value of three perpendicular measurements (4). At a tumor size of 1 to 1.5 cm, cisplatin treatment was started. Twenty-six rats received a cisplatin injection of 1 mg/kg BW twice a week. Ten rats concurrently received injections of 10  $\mu$ g Org.2766 four times per week, 10 rats concurrently received saline injections four times a week, whereas six rats received no treatment concurrently with the cisplatin injections. Six animals received no treatment at all. The tumor reduction was measured twice a week as described above. In the control group of rats that received no treatment, the tumor continued to grow. In all cisplatin-treated rats there was an almost complete tumor regression within 14 to 17 days (average 89% tumor load reduction). The results of the measurements of tumor size are listed in Table 1. Statistical analysis was by an analysis of variance. No significant differences in tumor regression were observed between any of the cisplatin-treated groups of animals, except for a larger regression at day 7 in the cisplatin/Org.2766-treated animals compared with cisplatin/saline-treated animals [ $F = 1.55$  (9, 9),  $P < 0.05$ ].

These results suggest that Org.2766 protects against the neurotoxic side-effects of cisplatin treatment in rats. The slowing of the HSNV which occurred in cisplatin/saline-treated rats was absent in cisplatin-treated rats that received concurrent injections of Org.2766. Org.2766 did not hamper the antitumor activity of cisplatin. This observation and the absence of serious toxic side effects of Org.2766 warrants the investigation of the ability of Org.2766 to counteract cisplatin neurotoxicity in cancer patients that are

treated with cisplatin-based chemotherapy. The human studies undertaken to date have been aimed mostly at reducing the nephrotoxic side effects of cisplatin treatment (9). As it becomes increasingly clear that the neurotoxic side effects of cisplatin are the major limiting factor to its therapeutic use, a substance that may reduce this neurotoxicity, might, in our opinion, prove to be of profound value and interest.

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