Evaluation of *cis*-Diamminedichloroplatinum (II) (Cisplatin) Neurotoxicity in Rats¹

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Evaluation of *cis*-Diamminedichloroplatinum (II) (Cisplatin) Neurotoxicity in Rats. DE KONING, P., NEIJT, J. P., JENNEKENS, F. G. I., AND GISPEN, W. H. (1987). *Toxicol. Appl. Pharmacol.* 89, 81-87. Cisplatin neurotoxicity was studied in rats by using electrophysiological and functional tests. The drug was injected intraperitoneally two times a week (1 mg/kg) up to a cumulative dose of 19.0 mg/kg body wt. At this dose the H-reflex-related sensory nerve conduction velocity was reduced. After cessation of drug administration, the H-reflex-related sensory nerve conduction velocity slowly improved. The motor nerve conduction velocity remained unaffected throughout the intoxication period and concentric needle investigation showed no signs of denervation. In addition, the sensorimotor response to a noxious stimulus presented locally to the foot sole was not affected by the cisplatin treatment. It is concluded that measurement of H-reflex-related sensory nerve conduction velocity is a useful and accurate tool in the evaluation of cisplatin neurotoxicity in rats. © 1987 Academic Press, Inc.

Cisplatin (*cis* - diamminedichloroplatinum (II)) is widely used as a cancer chemotherapeutic agent. The major dose-limiting factor is its nephrotoxicity. Other side effects are of gastrointestinal, hematologic, and neurologic origin (Van Hoff *et al.*, 1979). The neurotoxic side effects include peripheral neuropathy, retrobulbar neuritis, neurosensory hearing loss, and dorsal column degeneration. The cisplatin neuropathy is of a primarily sensory nature with an early decreased vibratory sensibility in toes, diminished proprioception, and loss of ankle jerks. The sensitivity to pain and temperature sense are relatively spared (Walsh *et al.*, 1982; Thompson *et al.*, 1984). Neurophysiological studies show decreased

sensory but normal motor nerve conduction velocities with occasional signs of denervation (Thompson *et al.*, 1984; Reinstein *et al.*, 1980).

The objective of the reported investigations was to find an animal model in which the neurotoxic side effects of cisplatin can be properly evaluated. Thus far this has proved to be difficult because of nephrotoxicity. However, nephrotoxicity can largely be overcome by adequate dosing schedules, hydration, and administration of furosemide (De Jong *et al.*, 1983; Tomiwa *et al.*, 1986; Ward *et al.*, 1977). Another major problem has been the selection of parameters that are representative measures for cisplatin neurotoxicity in an animal model (Rebert *et al.*, 1984). In the present study, we have measured in the rat the H-reflex-related sensory nerve con-

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duction velocity and motor nerve conduction velocity by means of transcutaneous stimulation of the sciatic nerve and by recording the H- and M-responses from the small muscles of the foot with surface electrodes. The M-response occurs in reaction to stimulation of the α -motor fibers while the H-response reflects stimulation of the afferent proprioceptive Ia fibers, which in the spinal cord monosynaptically excite α -motor neurons (Hoffman, 1918). The transcutaneous stimulation allows longitudinal assessment of cisplatin neurotoxicity in a given subject. In humans the H-reflex is usually recorded from the calf muscles, and to our knowledge there is only one report on the recording of H-reflexes from the muscles on the plantar surface of the foot (Wager and Buerger, 1974). There are more reports on the recording of F-responses from human foot muscles, which are responses due to antidromic conduction in motor axons (Mayer and Feldman, 1967). However, in rats the H-response can be easily recorded from the small muscles on the plantar surface of the foot (Stanley, 1981). There are three observations which provide evidence that the response that is recorded in combination with the M-response is an H-response and not an F-response. First, the H-response has a lower stimulation threshold than the M-response, whereas the F-response occurs only at supramaximal stimulation. Second, the H-response can be recorded with a larger amplitude than the M-response at low stimulus intensities. Third, the ratio $H_{\max}/M_{\max}$ is approximately 0.5, whereas the ratio $F_{\max}/M_{\max}$ is approximately 0.005. Furthermore, we studied the possible occurrence of denervation by means of concentric needle investigation of the calf muscles and assessed the responsiveness of the hindpaw to local noxious stimulation of the foot sole (De Koning *et al.*, 1986).

MATERIALS AND METHODS

Animals. Female Wistar rats from an inbred strain (TNO, Zeist, NL) weighing approximately 190–210 g at the onset of the experiments were used (age: 12–13

weeks). They were maintained on a 12-h dark–light cycle with the lights on beginning at 7:30 AM.

Cisplatin treatment. A cisplatin solution containing 25 mg cisplatin/50 ml solvent (Platinol, Bristol-Myers, E) was diluted with saline to a final concentration of 0.04 mg/ml. The animals received an intraperitoneal injection of cisplatin (1 mg/kg) twice a week (on Tuesday and Friday). Twenty minutes prior to the cisplatin administration, both cisplatin- and saline-treated rats received furosemide (12.5 mg/kg) subcutaneously (Lasix, Hoechst, FRG).

Electrophysiology. All electrophysiological examinations were carried out under general anesthesia (Hypnorm, Duphar, NL, containing fluanisone 10 mg/ml and phentanyl citrate 0.2 mg/ml, dose 0.8 ml/kg body wt, administered subcutaneously). To minimize effects of body temperature differences on conduction velocity, the animals were placed under a 40-W light bulb (at a distance of 25 cm) 5 min prior to testing. The entire recording procedure took only 10 min per animal and is described by Stanley (1981). In short, the tibial and sciatic nerves were stimulated at the ankle and sciatic notch, respectively, by a 0.1-msec square-wave pulse delivered by a Grass S8 stimulator and through a Grass stimulus isolation unit. The M- and H-responses (see below) were recorded from the small muscles on the plantar surface of the hindfoot by means of 7.0-mm surface recording electrodes. The rat was grounded by means of a 4-mm-thick copper wire which was wrapped around the ankle between the stimulation and recording points. After amplification the responses were recorded on a VUCO VKS 22-16 digital storage scope. Response latencies were de-

FIG. 1. Schematic illustration of the test model. The pictures show the M- and H-responses recorded from the small muscles of the foot by a surface electrode after stimulating at the sciatic notch (a) and ankle (b), respectively.

defined as the interval between stimulus artifact and the beginning of the deflection of the M- or H-response.

Upon stimulation of the mixed sciatic or tibial nerve, two responses occur. The M-response is a direct motor response with short latency due to stimulation of the α -motor fibers. The H-reflex is an indirect response with long latency due to stimulation of the afferent proprioceptive Ia fibers. The Ia fibers monosynaptically excite α -motor neurons in the spinal cord (Fig. 1). At lower stimulation intensities only the thicker afferent fibers are excited and only an H-reflex is elicited. At increasing stimulation intensities both H- and M-responses occur, while at repeated supramaximal stimulation the H-reflex is absent due to antidromic conduction in the motor axons.

The distance between the two stimulation points was measured over the skin with the hip and knee in a flexed position. Motor and H-reflex-related sensory conduction velocity were calculated as follows:

Motor conduction velocity

$$= \text{distance (sciatic notch - ankle)} / \text{latency}$$

$$(\text{M-response sciatic notch} - \text{M-response ankle})$$

and

H-reflex-related sensory conduction velocity

$$= \text{distance (sciatic notch - ankle)} / \text{latency}$$

$$(\text{H-response ankle} - \text{H-response sciatic notch}).$$

Signs of denervation (positive sharp waves and fibrillation potentials) were looked for by means of a concentric needle. We investigated the gastrocnemius and soleus muscles in all rats.

Noxious stimulation of the foot sole. The sensitivity to pain was assessed by applying a small electric current (100 μ A strength of current) to the foot sole through two stimulation poles (De Koning *et al.*, 1986). When the skin of the foot sole closes the electric circuit, healthy rats invariably withdraw their paw instantaneously.

Statistics. The results were analyzed using an analysis of variance with repeated measures.

Experiments. In the first experiment, rats ($n = 20$) were housed in single metabolism cages (UNO, NL) and their body weight, water intake, and urine production were assessed daily. Half of the rats were treated with cisplatin up to a cumulative dose of 13 mg/kg. The other half of the rats received saline injections ip. Neurophysiological examinations were performed on the day of cisplatin treatment, 2 hr prior to drug administration.

In the second experiment, rats ($n = 22$) were housed in macrolon cages (4 per cage, RUCO, NL) on sawdust. They had access to commercial rat chow and water *ad libitum*. Twelve rats were treated with cisplatin up to a cumulative dose of 19 mg/kg, while the other 10 rats received saline injections ip. Neurophysiological examinations were performed on the day prior to the start of cisplatin treatment and at various times throughout the in-

toxication period 2 hr prior to drug administration. After cessation of cisplatin treatment 4 animals from each group were neurophysiologically examined several times during a 9-week follow-up period.

In a third experiment, similar to the second experiment, neurophysiological examinations were performed only four times: at the start of the experiment and after a cumulative dose of cisplatin of 13, 16, and 19 mg/kg, respectively.

RESULTS

Metabolism and Health

After an initial reduction in weight (approx 10%) following the start of cisplatin administration, rats maintained their weight. As compared to saline-treated rats, rats receiving cisplatin had a reduced fluid intake (20 versus 30 ml/day) with a balanced urinary output indirectly indicating adequate kidney function.

In the first experiment (maximum cumulative dose of cisplatin 13 mg/kg body wt) 2 out of 10 rats in the cisplatin-treated group died. In the second experiment (maximum cumulative dose of cisplatin 19 mg/kg body wt) 4 out of 12 cisplatin-treated rats died. In the third experiment only 1 out of 12 cisplatin-treated animals died. At necropsy we found a large distension of the colon, especially in the cecum, with signs of dry peritonitis. The feces production had remained normal throughout the experiments. No diarrhea was observed. The kidneys looked normal macroscopically. Before dying most rats developed pallor, showed muscle wasting, and lost weight.

Electrophysiology

As already explained under Materials and Method, the H-reflex in healthy rats has a lower stimulation threshold than the M-response. In the first experiment, from a cumulative dose of cisplatin of 11 mg/kg onward, the H-reflex could be elicited only at stimulation intensities that also resulted in an M-response. At lower stimulation intensities the

FIG. 2. (a) The left part of the figure shows the time course of the H-reflex-related sensory nerve conduction velocity in the sciatic nerve during the period of drug administration. To the right the time course of the sensory conduction velocity is shown after cessation of drug administration. Solid line indicates mean value of cisplatin-treated animals (means $\pm$ SE, $n = 8$). Hatched line indicates mean values of saline-treated controls (means $\pm$ SE, $n = 10$). Arrow points indicate cisplatin administration (1 mg/kg body wt). (b) Mean values of motor conduction velocity during the period of cisplatin administration (means $\pm$ SE). (c) Mean values of the conduction in the proximal part of the sciatic nerve (including the dorsal root ganglion and the spinal cord) calculated as the difference between the H- and M-response latencies after stimulation at the sciatic notch (means $\pm$ SE).

H-reflex was unobtainable. At low stimulation intensities, however, the H-response could still be elicited with a higher amplitude than the M-response, excluding the possibility that we had recorded an F-response. During the 6-month follow-up period after cessation of cisplatin administration, in the cisplatin-treated rats the H-reflex remained at a stimulation threshold equal to or higher than that of the M-response.

The results of the second experiment are summarized in Figs. 2a–2c. Again, starting at a mean cumulative dose of cisplatin of 11 mg/kg, the H-reflex could no longer be elicited at a stimulation strength lower than that necessary to produce the M-response (Table 1). After a mean cumulative dose of 13 mg/kg the H-reflex-related sensory conduction velocity began to slow. This deterioration continued during subsequent administration of cisplatin. The slowing of the sensory con-

duction velocity was highly significant compared to the saline-treated rats ($F = 22.5$, $p < 0.001$). After cessation of cisplatin administration, four animals from each group (cisplatin or saline treated) were examined neurophysiologically several times during a 9-week follow-up period. The H-reflex-related sensory conduction velocity improved in the

TABLE 1

STIMULATION THRESHOLD (IN VOLTS) FOR GENERATING THE M-RESPONSE AND H-REFLEX ON STIMULATION AT THE ANKLE (MEAN $\pm$ SE, $n = 12$)

Cumulative dose cisplatin (mg/kg)	Stimulation threshold	
	M-response (volts $\pm$ SE)	H-reflex (volts $\pm$ SE)
0	3.0 $\pm$ 0.1	2.5 $\pm$ 0.1
11	3.1 $\pm$ 0.1	3.2 $\pm$ 0.1

formerly cisplatin-treated rats and after 9 weeks had reached control levels. However, the H-response still could not be elicited at stimulation strengths lower than those that also resulted in an M-response. As can be seen in Fig. 2c the conduction in the proximal part of the sciatic nerve (including the dorsal root ganglion and the spinal cord), calculated as the difference between the H- and M-response latencies after stimulation at the sciatic notch, was not affected. The motor nerve conduction velocity showed no slowing (Fig. 2b). Concentric needle investigation of the soleus and gastrocnemius muscles revealed no signs of denervation in the cisplatin-treated group of rats at the end of the intoxication period or at the end of the follow-up period.

In the third experiment, with a limited number of neurophysiological examinations, the H-reflex-related sensory nerve conduction velocity showed the same amount of slowing in the cisplatin-treated rats as in the second experiment.

Sensitivity to Pain

In both experiments the sensitivity to noxious stimulation of the foot sole remained intact in the cisplatin-treated animals.

DISCUSSION

Using the test model described in this paper, we were able to determine and quantify the neurotoxic side effects of cisplatin in a reliable manner. The noninvasive electrophysiological procedure allows the evaluation of the developmental and recovery phases of the neuropathy in the same animal. The administration of cisplatin at a dose of 1 mg/kg two times per week appeared to be an acceptable dose regimen, since a majority of rats survived and developed signs of a peripheral neuropathy during the intoxication period. The large distension of the colon found at necropsy might have resulted from an auto-

nomic neuropathy. Thus far, only one case of an autonomic neuropathy has been reported in humans (Rosenfeld and Broder, 1984). Repeated anesthesia may have caused the death of certain animals that were debilitated by cisplatin. This suggestion is supported by the observation that in the third experiment, with a limited number of neurophysiological examinations, only 1 out of 12 cisplatin-treated rats died. Thus, the times that rats undergo general anesthesia should be limited.

The first sign of neurotoxicity appeared to be a decreased sensitivity of Ia fibers to electrical stimulation. This pattern of reduced excitability is also reported in relation to lumbar root compression (Notermans and Vingerhoets, 1974). The subsequent slowing of the H-reflex-related sensory nerve conduction velocity on continued administration of cisplatin improved after cessation of drug administration, but the decreased excitability of the Ia fibers remained. Reports on the time course of polyneuropathy in humans after cessation of cisplatin therapy are contradictory. Some authors report an improvement, whereas others observed an initial worsening followed by an improvement and still others describe an ongoing polyneuropathy (Tompson *et al.*, 1984; Reinstein *et al.*, 1980; Roelofs *et al.*, 1984; Hadley and Herr, 1979). In human electrophysiological studies the first sign of cisplatin neurotoxicity is the prolongation of the sensory latency in the sural nerve (Tompson *et al.*, 1984; Reinstein *et al.*, 1980). Motor nerves are seldom affected and denervation potentials are rarely observed. In the present study the motor nerve conduction velocity was not affected and no denervation potentials were detected in the gastrocnemius and soleus muscles. As all cisplatin-treated rats reacted to a small electric current applied to the foot sole, it seems unlikely that the unmyelinated fibers conducting pain sensation were much impaired. This is in line with observations in humans in which sensitivity to pain is seldom affected during cisplatin therapy (Roelofs *et al.*, 1984). The time course of recovery and human and animal

histological studies are suggestive of degeneration and regeneration as causes of the observed slowing and subsequent improvement of the H-reflex-related sensory nerve conduction velocity. However, there are some other possibilities, such as slowing of conduction along individual fibers or an alteration in excitability as is suggested by the increased threshold for H-reflex generation. Histological studies in humans show that nerve fibers are affected more distally than proximally. A histological study in rats that had received chronic treatment with cisplatin revealed Wallerian degeneration in the sciatic nerve at all levels examined (Clark *et al.*, 1980). The total dose of cisplatin administered in that study, however, largely exceeded the one in the present study.

When these data are compared with previously reported control values (Stanley, 1981), two points deserve comment. First, the H-reflex-related sensory nerve conduction velocity and the motor nerve conduction velocity are about 10 m/sec faster for the weight class than those reported by Stanley (1981). This can partly be explained by the different rat strain. Sprague-Dawley rats are apparently heavier at the same age than Wistars used in this study. Therefore, in the same weight class, Wistar rats are older and have faster nerve conduction velocities. Second, there is a somewhat abrupt increase in conduction velocity in the control rats at 7 weeks. However, the overall increase in conduction velocities during the period of investigation (18 weeks) is of the same order of magnitude as that reported by Stanley (1981). Finally, the motor and H-reflex-related sensory nerve conduction velocities in the saline-treated rats, which also received furosemide, did not differ from those in untreated age-matched rats. It is concluded, therefore, that furosemide does not influence these conduction velocities.

In summary, the test model presented in this paper allows testing of various cisplatin treatment schedules for possible differences in neurotoxicity. Furthermore, using this test

model, we can investigate the possible efficacy of different substances to prevent or counteract cisplatin neurotoxicity.

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