Neuronal involvement in cisplatin neuropathy: prospective clinical and neurophysiological studies

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Although it is well known that cisplatin causes a sensory neuropathy, the primary site of involvement is not established. The clinical symptoms localized in a stocking-glove distribution may be explained by a length dependent neuronopathy or by a distal axonopathy. To study whether the whole neuron or the distal axon was primarily affected, we have carried out serial clinical and electrophysiological studies in 16 males with testicular cancer before or early and late during and after treatment with cisplatin, etoposide and bleomycin at limited (≤400 mg/m² cisplatin), conventional (~400 mg/m² cisplatin) or high (>400 mg/m² cisplatin) doses. At cumulative doses of cisplatin higher than 300 mg/m² the patients lost distal tendon and H-reflexes and displayed reduced vibration sense in the feet and the fingers. The amplitudes of sensory nerve action potentials (SNAP) from the fingers innervated by the median nerve and the dorsolateral side of the foot innervated by the sural nerve were 50–60% reduced, whereas no definite changes occurred at lower doses. The SNAP conduction velocities were reduced by 10–15% at cumulative doses of 400–700 mg/m² consistent with loss of large myelinated fibres. SNAPs from primarily Pacinian corpuscles in digit 3 and the dorsolateral side of the foot evoked by a tactile probe showed similar changes to those observed in SNAPs evoked by electrical stimulation. At these doses, somatosensory evoked potentials (SEPs) from the tibial nerve had increased latencies of peripheral, spinal and central responses suggesting loss of central processes of large dorsal root ganglion cells. Motor conduction studies, autonomic function and warm and cold temperature sensation remained unchanged at all doses of cisplatin treatment. The results of these studies are consistent with degeneration of large sensory neurons whereas there was no evidence of distal axonal degeneration even at the lowest toxic doses of cisplatin.

Keywords: testicular cancer; cisplatin neurotoxicity; neurophysiology; nerve conduction; evoked potential

Abbreviations: DP = dorsum of the foot; LM = lateral malleolus; MC = midcalf; SNAP = sensory nerve action potentials

Introduction

Cis-diamminedichloroplatinum II (cisplatin) (Lippard, 1982; Daugaard et al., 1987, 1990) is the drug of choice in the treatment of germ cell tumours and is used in the treatment of a variety of other solid tumours. Its neurotoxic effect (Roelofs et al., 1984; Thompson et al., 1984; Boogerd et al., 1990) is well established and constitutes a major limiting factor in the treatment of various malignancies. Whereas sensory fibres primarily are affected, it is not certain whether the whole sensory neuron or the distal part of the sensory axon is first involved in the pathological process. In most patients the sensory symptoms associated with cisplatin treatment are first localized to the distal parts of the lower limbs and then to the upper limbs in a ‘stocking-glove’ distribution, consistent with a length dependent dying-back neuropathy and claimed to be due to distal degeneration of sensory axons (Gastaut and Pellisier, 1985; Hansen et al., 1989). This question is important since distal axonal degeneration could be reversible at discontinuation of treatment whereas degeneration of the sensory cell body would be expected to cause irreversible axonal loss (Asbury, 1987). Improvement of symptoms has been reported in a number of patients after...
discontinuation of treatment (Hadley and Herr, 1979; Thompson et al., 1984), though it has not been documented whether this recovery is associated with changes in axonal function. Measurements in patients treated with cisplatin have shown that the compound is concentrated at higher levels in dorsal root ganglion cells than in peripheral nerve or in the central nervous system (Thompson 1984; Krarup-Hansen 1998; Fischer et al., 1999), and cisplatin is now considered to cause apoptosis of sensory neurons (Gill and Windebank, 1998; Fischer et al., 2001; Bowers et al., 2002; McDonald and Windebank, 2002; Peltier and Russell, 2002). Thus, the most likely cause of peripheral nerve damage is loss of function of the whole sensory neuron consistent with our previous retrospective study (Krarup-Hansen et al., 1993).

Nevertheless, evolving damage during treatment may initially be located at the distal sensory axon or include the whole neuron (Windebank et al., 1994). To investigate this question we have followed males with testicular cancer during chemotherapy to localize the first abnormalities along the peripheral and central sensory axons. Sensory conduction studies were carried out at different distal sites in the upper and lower limbs, and in order to examine the possibility that the very distal end of the sensory axon was involved early, the responses of touch receptors to tactile stimulation were compared to the nerve responses obtained by electrical stimulation of more proximal nerve segments. In addition, abnormalities in small and large nerve fibres were compared by testing vibratory and temperature perception. The findings confirmed that clinical as well as pathophysiological changes only developed in patients treated with cumulative doses of more than 300 mg/m^2 body surface area and furthermore showed that the entire long, large sensory neurons were involved consistent with neuronal degeneration. Brief reports have been published (Krarup-Hansen et al., 2004; Krarup et al., 2005).

### Material and methods

Sixteen men, aged 23–46 years, with testicular cancer [13 with embryonic carcinoma and 3 with seminoma, stages 2–3 (Table 1)] were followed during treatment with cisplatin, etoposide and bleomycin. The study was approved by the institutional ethical committee. One additional patient filled enrolment criteria but refused follow-up studies, and nine patients could not participate since this would have caused unacceptable delay in treatment. None of the patients had reduced nutritional status or were systemically affected by the malignant disease. None had laboratory signs of diabetes mellitus. One patient (#3) with a subclinical sensorimotor neuropathy on electrophysiological examination before chemotherapy was included in the study; a sural nerve biopsy was carried out 9 months after the final fourth dose of chemotherapy (see Table 1). One patient (#10) informed that he had had high alcohol consumption for many years; neurological and electrophysiological examination was normal before start of chemotherapy. One patient (#1) had mediastinal metastases only partially affected by chemotherapy; he later died from his testicular cancer.

The patients were referred to the Oncology Clinic, Rigshospitalet consecutively. Enrolment criteria included age ≥18 years, with UICC stage 2 or higher germ cell (testicular) cancer, and without symptoms or signs of neurological or neuromuscular disease. The patients were planned to start intended curative antineoplastic treatment with cisplatin-based chemotherapy immediately. The patients had not received cytostatic treatment before enrolment and were assigned for standard intravenous combination chemotherapy according to

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**Table 1 Clinical and treatment data in 16 males with testicular cancer**

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<th>Pt. no.</th>
<th>Initials</th>
<th>Age (years)</th>
<th>Surface (m²)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Diagnosis of cancer</th>
<th>Stage of disease</th>
<th>No. of courses</th>
<th>Planned dose (mg/m²)</th>
<th>Cumulative dose of cisplatin (mg/m²)</th>
<th>No. of courses of PEB</th>
<th>Dose intensity of cisplatin (mg/(m² × wk))</th>
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*Cisplatin (P), etoposide (E) and bleomycin (B). CD, conventional dose; HD, high dose.*
established criteria. Three low-risk patients were treated with three courses of conventional dose (see subsequently), 11 intermediate-risk patients with four courses of conventional dose, and one high-risk patient was treated with three high doses and in another high-risk patient with one added conventional dose course [Table 1 (Germ Cell Consensus Classification, 1997)]. Patients with abnormally high tumour markers (α-fetoprotein, β-chorionic gonadotrophin) at pretreatment examination continued the chemotherapy without dose modification for one course after normalization of markers. According to recovered haematological parameters, the chemotherapy courses were repeated with intervals of 3 weeks.

The conventional dose course (Chaudhary and Haldas, 2003) consisted of cisplatin 20 mg/m² body surface area on days 1–5, etoposide 100 mg/m² on days 1–5, and during the first three courses bleomycin 15,000 IU/m² on days 2, 9 and 16; during the fourth course the same schedule was followed with reduced bleomycin to 5,000 IU/m². No patient received more than four courses of bleomycin containing chemotherapy.

Thus, low-risk patients were treated with approximately cumulated doses of 300 mg cisplatin/m², intermediate-risk patients 400 mg cisplatin/m² and high-risk patients 600 mg cisplatin/m² or more. Response to chemotherapy was evaluated according to WHO criteria. Patients with residual disease assessed by CT scan of thorax and abdomen at the end of chemotherapy underwent surgical metastectomy. Systemic toxicity was graded according to WHO criteria (Postma and Heimans, 2000).

**Longitudinal studies**

All patients had standard pretreatment kidney and lung tests and follow-up studies were carried out throughout the treatment to ensure toxicity did not exceed acceptable levels. These included blood counts, blood glucose, lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, plasma bilirubin, coagulation factors, plasma electrolytes (Na⁺, K⁺, Mg²⁺, Ca²⁺), urea, creatinine, albumin, protein, cobalamin, total cholesterol, high- and low-density lipoprotein cholesterol, triglyceride, IGG, IGA, IGM, DNA-anti-nuclear-antibody, tumour markers in serum and low-density lipoprotein cholesterol, triglyceride, IGG, IGA, creatinine, albumin, protein, cobalamin, total cholesterol, high- and low-density lipoprotein cholesterol, triglyceride, IGG, IGA, IGM, DNA-anti-nuclear-antibody, renal Cr⁵¹EDTA-clearance and lung function test.

To evaluate neurotoxicity, nerve conduction studies, somatosensory and brainstem auditory evoked potentials, autonomic studies and quantitative sensory evaluation were planned before, during and after chemotherapy (Table 2). A standardized neurological examination was obtained to screen for pretreatment neurological disorders including motor and sensory function and was repeated as indicated below.

The changes in clinical examination and electrophysiological parameters were based on findings before administration of antineoplastic medication. The first study could, however, only be scheduled before the first treatment course in 10 patients, and had to be delayed until 1–8 days before the second treatment in six patients who at this time had received 89–113 mg/m² in five and 200 mg/m² in one patient who was in urgent need for chemotherapy. A second and final examination was carried out at 114–224 days after the last course in four patients (three with conventional dose and one with high dose) who received three courses of treatment. A second study was carried out 16–24 days after the third treatment in eight of 12 patients who received four courses, and in all 12 patients a final third study was carried out 85–270 days after the final course (Table 2).

**Studies of peripheral nerve function**

Sensory function was studied in the median and sural nerves including conventional nerve conduction studies and investigation of sensory receptor function. Motor fibres of the median and peroneal nerves and H-reflexes from the soleus muscles were studied. Autonomic and unmyelinated or small sensory fibres were studied. The methods applied were described in detail in a previous study of patients with testis cancer (Krarup-Hansen et al., 1993).

**Quantitative sensory evaluation**

Vibratory perception threshold (VPT) at 100 Hz was determined on the big toe, the dorsum of the foot and on the tip of digit 3 using a hand-held vibrator with adjustable indentation depth (Nielsen, 1972). Warm and cold perception was assessed with a Peltier element applied on the dorsum of the hand and the plantar surface of the foot (Fowler et al., 1987). Touch, position and pin-prick sensation were examined qualitatively by conventional means.

**Sural nerve conduction studies**

The distal and intermediate segments of the sural nerve from the dorsum of the foot (DP) to the lateral malleolus (LM) and to the midcalf (MC) were studied with the patient placed on the side and the leg placed on a firm pillow. Electrical and tactile stimuli were applied to the DP, and the compound sensory action potentials (SNAP) were recorded at LM and MC (Fig. 1). The electrical stimuli (0.2 ms in duration) were applied to the nerve through needle electrodes, and the tactile stimuli through a probe at DP (Buchthal, 1982a, b). The repetitive tactile stimulus was a 200 μm indentation with an indentation velocity of 400 μm/ms (Krarup-Hansen et al., 1993; Krarup and Trojaborg, 1994; Krarup, 2004). The SNAP was recorded through needle electrodes with a 3 mm bared tip placed close to the nerve and referenced to another needle electrode placed at a transverse distance of 3–4 cm, amplified (Dantec 15C02, 200–4000 Hz), digitized at a sampling interval of 20 μs and 500–1000 responses were averaged (Nicolet 20 Pro) for off-line analysis. In addition electrical stimuli were applied to the nerve at LM and the SNAP was recorded at MC through the same electrodes.

**Median nerve conduction studies**

With the arm placed on a firm padded arm board and the palm facing up, sensory fibres from digits 1 and 3 were studied by applying electrical stimuli (0.2 ms) through surface ring-electrodes placed at the distal phalanx of digit 1 and the middle phalanx of digit 3, and through EEG-needle electrodes placed on each side of the tip of digit 3. The tactile probe was applied at the same site, the tip of digit 3. The SNAP's evoked by electrical and tactile stimulation were recorded through needle electrodes placed close to the median nerve at wrist and elbow following the same procedure as at the sural nerve (cf. above) (Fig. 2). Motor fibres were studied by applying electrical stimuli at wrist and elbow, and the CMAP was recorded through surface electrodes over APB, amplified (Dantec, 10Hz–10kHz), digitized and stored for off-line analysis.
Peroneal nerve conduction studies
Electrical stimuli (0.2 ms in duration) were applied to the deep peroneal nerve through surface electrodes at the ankle and the fibular head, and the CMAP was recorded through surface electrodes in a belly–tendon montage over the extensor digitorum brevis muscle (EDB) and amplified (Dantec, 10 Hz–10 kHz).

H-reflex studies
With the patient in a prone position, and the ankles elevated by 20 cm, electrical stimuli (duration 1 ms) were applied at threshold for the CMAP to the tibial nerve in the popliteal fossa through surface electrodes; the CMAP was recorded through surface electrodes over the soleus muscle referenced to another surface electrode placed over the Achilles tendon, and amplified (Dantec, 10 Hz–10 kHz). The stimulus strength was gradually increased to supramaximal levels in order to distinguish an H-reflex from the F-wave.

Evoked potentials
BAEP were elicited from each ear in turn using a stimulus of 100 dB applied at a rate of 10 Hz (Pedersen and Trojaborg, 1981); averaged responses to 2048–4096 stimuli were recorded through a Cz–mastoid montage. The mean value of the latencies from the right and left sides was compared with control values obtained in the laboratory.

SEPs following tibial nerve stimulation at the medial malleolus were recorded through needle electrodes placed at the popliteal fossa or the gluteal fold, at vertebra Th12, and at the scalp in a Cz–Fz montage (Trojaborg and Petersen, 1979). Conduction velocities along peripheral nerves and latencies of spinal and cortical responses were averaged on the two sides.

Autonomic studies
Autonomic function (Ewing and Clarke, 1982) was tested by recording the heart rate during deep breathing at a rate of 6/min and after Valsalva manoeuvre, and the values were compared with age-matched controls in the laboratory.

Electrophysiological parameters
The latency of the electrically evoked SNAP was measured from the onset of the stimulus to the first positive peak (Figs 1 and 2). The distal conduction velocity of the electrically evoked action potential was calculated from the conduction distance and the latency. The latency of the tactile response was measured from the time when the tactile stimulus had reached 10% of maximum to the first positive peak of the evoked SNAP (Loewenstein, 1971; Krarup and Trojaborg, 1994). The latency of the electrically evoked action potential at the dorsolateral side of the foot or the tip of digit 3 was subtracted from the latency of the tactile response evoked at the same sites in order to estimate the delay at the receptor. The SNCV of both the electrically evoked and the tactile SNAPs between the recording sites was calculated from the conduction time. Conventional methods were used to measure MNCV.

The amplitude of the SNAP was measured peak-to-peak; and that of the CMAP from the height of the negative phase.

The findings in the median, peroneal and sural nerves were compared with those obtained in 22 normal males (Krarup-Hansen et al., 1993) and the normal age-matched material used at the laboratory (Rosenfalck and

<table>
<thead>
<tr>
<th>Stage of disease</th>
<th>Number of patients</th>
<th>Cumulative dose (mg/m²)</th>
<th>Number of NCS per patient</th>
<th>Number of NCS before second treatment</th>
<th>Number of NCS with pretreatment NCS</th>
<th>Number of NCS at first NCS</th>
<th>Number of NCS at second NCS</th>
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<td>24–43</td>
</tr>
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</table>

CD, conventional dose; HD, high dose; NCS, nerve conduction study; ND, not done.
Rosenfalck, 1975; Trojaborg et al., 1992; Horowitz and Krarup, 1992). The SEPs were compared to 38 normal males, aged 26–44 years, examined in the laboratory.

Statistical analysis
The statistical procedures are described at the appropriate site. Significance levels of 0.05 were used.

Results
Grouping of findings according to cumulative doses of cisplatin
To facilitate comparison of the effects of antineoplastic treatment on different clinical and electrophysiological parameters, the patients were grouped in three treatment ranges, Group 1 (0–200 mg/m² that included 10 patients studied before treatment and 6 patients studied after the first and before the second course of chemotherapy), Group 2 (286–305 mg/m², mean SEM 297 ± 2 mg/m², n = 11), and Group 3 (385–711 mg/m², 436 ± 27 mg/m², n = 13).

The renal clearance decreased by 12% during treatment but none of the patients developed renal failure to the extent that may cause a neuropathy. The tumour markers normalized following chemotherapy. One patient (#10) received magnesium supplement due to low S-Mg^{++}, which has been correlated to neuropathy during cisplatin chemotherapy (Ashraf et al., 1990).

Neurological examination
At the start of treatment none of the patients had neuromuscular complaints or had a history of Raynaud phenomenon. The pretreatment neurological examination (Group 1) showed normal tendon reflexes and vibration perception threshold (Table 3). In Group 2 patients, one patient complained of cold feet, one of paraesthesiae of the hands, and one of Lhermitte’s sign (Dewar et al., 1986). In Group 3 patients, five complained of sensory symptoms including painful hands and feet in cold weather, and at neurological examination eight patients had sensory loss to touch and unsteady gait. Vibratory perception and reflexes showed dose dependent abnormalities (see Table 3).

One patient (#3) showed subclinical reduction of motor and sensory conduction velocities but normal amplitudes of the evoked responses in median, peroneal and sural nerves on the first examination 2 days before start of chemotherapy, and he developed pronounced symptoms of sensory loss in connection with cisplatin treatment (402 mg/m²). A sural nerve biopsy was carried out 9 months after the fourth treatment and showed loss of large myelinated fibres and fibre regeneration in a patchy distribution with some
fascicles showing loss of fibres whereas others were normal. Some vessels showed mononuclear infiltration, suggesting that the axonal degeneration and regeneration had a vasculitic cause.

**Reflex findings, sensory examination and autonomic function during treatment**

**Tendon and H-reflexes**

All 16 patients in Group 1 had normal tendon reflexes (Table 3). The knee and ankle jerks were normal in 91% of patients in Group 2; the knee jerks were present in 69% in Group 3, whereas the ankle jerks were lost in all patients in this group. H-reflexes recorded at the soleus muscle were present in all Group 1 and in 9 of 11 Group 2 patients, whereas they could only be recorded in one of 13 Group 3 patients (Table 3); the presence of Achilles tendon reflexes and H-reflexes was highly correlated ($\chi^2 = 30.79, P < 0.0005$). In contrast, F-waves to the soleus muscle were present in all tested patients, and the mean latency was $3 \pm 2\% \ (n = 12)$ longer in Group 3 than in Group 1 (not significant).

**Vibratory and temperature perception thresholds**

Vibratory perception threshold (VPT) examined at the tip of toe 1, at the DP, and at the tip of digit 3 did not differ significantly in Groups 1 and 2 but deviated significantly ($P < 0.0005$, ANOVA) from both in Group 3 patients (Table 3). In Group 3, the average VPT compared to Group 1 increased by $250 \pm 46\%$ at digit 3, by $425 \pm 55\%$ at DP and by $331 \pm 35\%$ at toe 1.

The thresholds to cold and warm sensation at the hand and foot were increased in one patient before treatment but normalized after the third treatment according to published normal limits (Fowler et al., 1987). The thresholds were borderline in another three patients in Group 1. The thresholds remained unchanged at all doses of treatment (Table 3).

**Autonomic function testing**

The changes in heart rate during deep breathing at six breaths per minute and after Valsalva manoeuvre remained normal at all doses of treatment (Table 3).

**Amplitudes and conduction velocities of sensory responses**

**Sensory responses evoked by electrical stimulation**

Sural nerve SNAPs evoked at the LM and recorded at the MC (Fig. 1A) had normal amplitudes in Group 1 patients ($24 \pm 2\mu V$, mean $\pm$ SEM). Figure 4A illustrates the
amplitudes of sural nerve SNAPs from the DP as function of the cumulative dose of cisplatin and shows that there was no significant difference at doses of less than about 300 mg/m², whereas the amplitudes at higher doses decreased. Figure 4B illustrates the relative changes in amplitudes normalized to the first examination set at 100%. This was justified, since none of the studies of sensory nerves showed significant differences (Mann–Whitney U) between the SNAPs recorded before any treatment was given (e.g. sural nerve from DP to LM, 12.8 ± 1.6 μV, mean ± SEM, n = 10), compared with those obtained after the first treatment (11.8 ± 4.4 μV, n = 6).

The percentage changes in amplitudes of the sural and median nerve SNAPs (Fig. 5A and B) from DP, LM, digit 3 and the tip of digit 3 were slight and variable (P > 0.1, ANOVA) at doses up to 300 mg/m² in Group 2 compared with Group 1; the amplitude of the SNAP from digit 1 was slightly reduced in Group 2 compared with Group 1 patients (P < 0.05). The amplitudes at all sites were significantly reduced by 45–65% at higher doses in Group 3 (P = 0.01–0.001, ANOVA, Tukey post hoc). The reduction in amplitudes did not differ significantly (P > 0.5) when compared in the median and sural nerves (ANOVA, repeated measures).

Compared with controls in the sural nerve, one of 16 patients deviated from normal in Group 1, two of 11 in Group 2, and 11 of 13 in Group 3 with a strong correlation between the cumulative dose of cisplatin and the number of abnormal observations (χ² = 22.517, P < 0.0005). Similarly, in the median nerve, the SNAP amplitudes from digits 1 and 3 were normal in patients in Group 1, normal in all patients except two from digit 1 in Group 2, and it deviated from normal in 3–4 of 9–10 patients in Group 3 (χ² = 6.9–8.2, P < 0.03).

The SNCVs of both the sural and the median nerves remained unchanged in Group 2 compared with Group 1 but decreased by 8–13% in Group 3 (P < 0.02–0.0001, ANOVA, Tukey post hoc).

**Table 3** Tendon reflexes, H-reflexes, VPT, temperature threshold and autonomic testing in cisplatin treatment

<table>
<thead>
<tr>
<th>Tendon Reflexes</th>
<th>Group 1 (0–200 mg/m²) n = 16</th>
<th>Group 2 (286–305 mg/m²) n = 11</th>
<th>Group 3 (385–711 mg/m²) n = 13</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patellar reflex</td>
<td>Present 16, absent 0</td>
<td>Present 10, absent 1</td>
<td>Present 9, absent 4</td>
<td>χ² = 4.26, P = NS</td>
</tr>
<tr>
<td>Achilles reflex</td>
<td>Present 16, absent 0</td>
<td>Present 10, absent 1</td>
<td>Present 0, absent 13</td>
<td>χ² = 36.00, P &lt; 0.0005</td>
</tr>
<tr>
<td>H-reflex</td>
<td>Present 15, absent 0</td>
<td>Present 9, absent 2</td>
<td>Present 1, absent 12</td>
<td>χ² = 27.88, P &lt; 0.0005</td>
</tr>
<tr>
<td>VPT at toe 1</td>
<td>7.0 ± 0.8</td>
<td>8.9 ± 10†</td>
<td>22.6 ± 2.7†</td>
<td>ANOVA, P &lt; 0.0005</td>
</tr>
<tr>
<td>VPT at DP†</td>
<td>5.5 ± 0.7</td>
<td>7.2 ± 0.8†</td>
<td>22.7 ± 2.5†</td>
<td>ANOVA, P &lt; 0.0005</td>
</tr>
<tr>
<td>VPT at digit 3‡</td>
<td>3.7 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>8.7 ± 1.4</td>
<td>ANOVA, P &lt; 0.0005</td>
</tr>
<tr>
<td>Warm perception</td>
<td>1.5 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>ANOVA, NS</td>
</tr>
<tr>
<td>Warm perception</td>
<td>2.0 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>2.5 ± 0.5</td>
<td>ANOVA, NS</td>
</tr>
<tr>
<td>Cold perception</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>ANOVA, NS</td>
</tr>
<tr>
<td>Cold perception</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>ANOVA, NS</td>
</tr>
<tr>
<td>RR-interval</td>
<td>22 ± 3§</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
<td>ANOVA, NS</td>
</tr>
<tr>
<td>Pulse change</td>
<td>43 ± 5§</td>
<td>46 ± 4</td>
<td>51 ± 4</td>
<td>ANOVA, NS</td>
</tr>
</tbody>
</table>

*Vibration perception threshold (VPT) at toe 1, †at the dorsolateral side of the foot, DP ‡at digit 3, ‡the VPT in Group 2 did not differ significantly from Group 1, ‡the VPT in Group 3 differed significantly from Group 1 and Group 2, ‡1 patient was not studied.

The SNCVs of both the sural and the median nerves showed significant differences (Mann–Whitney U) between the cumulative dose of cisplatin and shows that there was no significant difference at doses of less than about 300 mg/m², whereas the amplitudes at higher doses decreased. Figure 4B illustrates the relative changes in amplitudes normalized to the first examination set at 100%. This was justified, since none of the studies of sensory nerves showed significant differences (Mann–Whitney U) between the SNAPs recorded before any treatment was given (e.g. sural nerve from DP to LM, 12.8 ± 1.6 μV, mean ± SEM, n = 10), compared with those obtained after the first treatment (11.8 ± 4.4 μV, n = 6).

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The SNCVs of both the sural and the median nerves remained unchanged in Group 2 compared with Group 1 but decreased by 8–13% in Group 3 (P < 0.02–0.0001, ANOVA, Tukey post hoc).

**Distal compared with more proximal sites of stimulation**

The percent changes in amplitudes of the SNAPs did not differ significantly at the different sites of stimulation in the foot or the fingers in Group 2 or Group 3 measurements (P > 0.1, ANOVA, repeated measures, Friedman). Similarly, when comparing the distal and the more proximal SNCVs from the side of the foot or from the fingers, the reduction that occurred in Group 3 was 10 ± 1% along the distal segment and 9 ± 1% along the proximal segment (n = 42, paired t-test, all studies pooled, P < 0.3) indicating that conduction along still functional fibres was not more reduced distally than proximally.

**Sensory nerve responses evoked by tactile stimulation**

Tactile responses were obtained by stimulation at DP (Fig. 1) and the tip of digit 3 (Fig. 2) and were compared...
with the responses evoked at the same site by applying supramaximal electrical stimuli, which activate a more proximal myelinated part of nerve fibres. It is apparent that the tactile responses, particularly when evoked by stimulation at the DP, were more dispersed and polyphasic than the SNAPs evoked by electrical stimulation (Figs 1 and 2). In Group 1 patients, the mean amplitude of the SNAP evoked at DP was 0.34 ± 0.02 mV and it was almost three times higher at digit 3, 0.82 ± 0.17 mV (P = 0.001, Wilcoxon), corresponding to 2–15% of the SNAP evoked by electrical stimulation (Figs 1 and 2) in agreement with previous studies (Krarup-Hansen et al., 1993; Krarup and Trojaborg, 1994; Simonetti et al., 1998). As a result of the mechanoreceptor delay, the latency of the tactile response from DP was 1.0 ± 0.1 ms longer than the latency at electrical stimulation. The delay was 40% longer (P < 0.005, Wilcoxon) at the foot than at digit 3 (0.6 ± 0.05 ms), which corresponded closely to normal subjects studied previously (Krarup and Trojaborg, 1994). The SNCVs of the tactile response from DP was 52.6 ± 1.6 m/s and of the electrical SNAPs 54.1 ± 1.3 m/s; from digit 3 the SNCV of the tactile SNAPs was 63.8 ± 2.0 m/s and of the electrical SNAP 64.3 ± 1.6 (P > 0.3, Wilcoxon) indicating that the responses were generated by the largest sensory fibres in the nerve.

**The effect of cisplatin on tactile responses**

Compared with Groups 1 and 2, the tactile responses in Group 3 decreased by 17 ± 12% from DP and by 50 ± 6% from digit 3 (Table 4). The responses from DP were smaller and more difficult to measure accurately than those from digit 3 which may account for the larger effects of cisplatin on amplitudes of SNAPs from the digit than from the foot. Nevertheless, the percent changes in the amplitudes of the SNAPs evoked by tactile and by electrical stimulation were correlated (P < 0.01, Fig. 6). Moreover, the percent changes in the amplitudes of tactile responses were significantly correlated with the percent increase in vibratory perception thresholds at the corresponding site at the foot and digit 3 (P < 0.05). The latencies of tactile responses were prolonged by 15–20% and the SNCVs were reduced by 5–10% from the foot and the finger in Group 3 compared with Groups 1 and 2 (Table 4), and the changes did not differ significantly when responses evoked by tactile and electrical stimulation were compared. The mechanoreceptor delay at the foot and the finger was prolonged by 30–50% in Group 3.
patients (Table 4). This prolongation was significant at the digit ($P < 0.001$) but barely at the foot ($P < 0.07$).

**Motor conduction studies**

The amplitudes and conduction velocities of CMAP of the peroneal and median nerves remained normal following treatment with cisplatin. Additionally the F-wave latencies of the peroneal and median nerves remained normal following treatment with cisplatin. Additionally the F-wave latencies of the peroneal and median nerves remained normal following treatment with cisplatin.

**Evoked potentials**

The BAEP latencies of the peripheral (PI) and central responses (PIII, PV) did not change over the course of the study.

**Sensory evoked potentials**

The tibial nerve SEP was slowed in patients treated with $>300$ mg/m$^2$ of cisplatin whereas the changes at lower doses were slight and variable. At a cumulative dose of $>300$ mg/m$^2$ in Group 3, the latency of the peripheral response recorded at the popliteal fossa or the gluteal fold was prolonged by $23 \pm 4\%$ ($P < 0.001$), that of the spinal response by $12 \pm 1\%$ ($P < 0.001$), that of the cortical onset by $18 \pm 4\%$ ($P < 0.001$) and of the P40 by $16 \pm 3\%$ ($P < 0.001$) and the central conduction time by $14 \pm 5$ ($P < 0.002$).

**Discussion**

Our study has as its main objective establishment of the initial location of axonal dysfunction in cisplatin induced neurotoxicity, which until now has remained ambiguous. We followed 16 patients with metastatic testicular cancer conventionally treated with cisplatin, etoposide and bleomycin in serial studies to determine the primary axonal abnormalities at the lowest toxic doses of cisplatin. Cisplatin was given in cumulative doses of 286–711 mg/m$^2$.

Table 4 Tactile compared with electrical responses in the sural and median nerves during treatment with cisplatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Statistics (ANOVA on percent deviations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP – LM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tact (ampl.), µV</td>
<td>0.42 ± 0.04 (16)</td>
<td>0.46 ± 0.05 (11)</td>
<td>0.33 ± 0.05 (13)</td>
<td>≥0.1</td>
</tr>
<tr>
<td>Electr (ampl.), µV</td>
<td>12.4 ± 1.8 (16)</td>
<td>11.0 ± 1.7 (11)</td>
<td>4.9 ± 0.9 (13)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tact (lat), ms</td>
<td>3.0 ± 0.1 (16)</td>
<td>3.2 ± 0.1 (11)</td>
<td>3.6 ± 0.2 (12)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Electr (lat), ms</td>
<td>2.0 ± 0.1 (16)</td>
<td>2.1 ± 0.1 (11)</td>
<td>2.4 ± 0.1 (13)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lat diff, ms</td>
<td>1.0 ± 0.1 (16)</td>
<td>1.1 ± 0.1 (11)</td>
<td>1.3 ± 0.2 (12)</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>Tact (SNCV), m/s</td>
<td>53 ± 2 (16)</td>
<td>52 ± 2 (11)</td>
<td>46 ± 2 (12)*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Electr (SNCV), m/s</td>
<td>54 ± 1 (16)</td>
<td>52 ± 1 (11)</td>
<td>47 ± 2 (13)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digit 3 – wrist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tact (ampl.), µV</td>
<td>0.88 ± 0.17 (14)</td>
<td>0.82 ± 0.12 (11)</td>
<td>0.35 ± 0.05 (10)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Electr (ampl.), µV</td>
<td>12.7 ± 2.5 (14)</td>
<td>11.4 ± 1.8 (11)</td>
<td>3.4 ± 0.5 (10)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tact (lat), ms</td>
<td>4.5 ± 0.1 (14)</td>
<td>4.7 ± 0.1 (11)</td>
<td>5.3 ± 0.1 (10)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Electr (lat), ms</td>
<td>3.9 ± 0.1 (14)</td>
<td>4.0 ± 0.1 (11)</td>
<td>4.4 ± 0.1 (10)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lat diff, ms</td>
<td>0.6 ± 0.05 (14)</td>
<td>0.7 ± 0.1 (11)</td>
<td>0.9 ± 0.1 (10)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tact (SNCV), m/s</td>
<td>64 ± 2 (14)</td>
<td>61 ± 2 (11)</td>
<td>60 ± 3 (10)*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Electr (SNCV), m/s</td>
<td>64 ± 1 (14)</td>
<td>63 ± 2 (11)</td>
<td>59 ± 2 (10)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean ± SEM are indicated in columns, number of patients in parenthesis. The findings in the sural nerve from the dorsolateral side of the foot (DP) to the lateral malleolus (LM) in the top part of the table and in the median nerve from digit 3 in the bottom part.

*Group 3 differed significantly, $P < 0.05$ from Groups 1 and 2, Tukey post hoc test.

Fig. 6 Relation between the relative changes (percent) in amplitudes of SNAP evoked by tactile and electrical stimulation in the sural (filled symbols, full line) and the median (open symbols, dashed line) nerves.
before treatment, and a sural nerve biopsy after treatment showed patchy loss of myelinated fibres, signs of regeneration and inflammation suggestive of a vasculitic cause of neuropathy. The patient did not have other clinical or laboratory signs of inflammation, and the cause for the neuropathy remained speculative. As a painter he had been exposed to solvents but there was no information of exposure to hexacarbons, which cause a symmetrical neuropathy. Paraneoplastic neuropathy was a possible cause though it is very rare in testicular cancer (McLeod et al., 1984); it may have a vasculitic like appearance (McLeod, 1993; Smitt and Posner, 1998). One other patient had a history of high alcohol consumption but had no pretreatment clinical or electrophysiological evidence of neuropathy. The neuropathy after cisplatin treatment (cumulative doses of 402 and 388 mg/m², respectively) in these two patients was pronounced. Patients with pre-existing peripheral nerve disorder may be particularly vulnerable to the neurotoxic effect of cisplatin (Bokemeyer et al., 1996; Quasthoff and Hartung, 2002; Chaudhary and Haldas, 2003).

**Distribution of involvement in cisplatin neuropathy**

While our study showed reduction of SNAP amplitude at cumulative doses of cisplatin of more than 300 mg/m² and confirmed previous prospective studies (Cowan et al., 1980; Roelofs et al., 1984; Thompson et al., 1984; Daugaard et al., 1987; Boogerd et al., 1990), reduction of SNAPs recorded at conventional sites cannot distinguish between distal axonopathy and neuronal cell body loss. Some studies have concluded that the distal stocking-glove distribution of sensory loss together with reduced amplitudes of SNAPs after cisplatin treatment indicated a distal axonopathy of ‘dying-back’ type (Gastaut and Pellisier, 1985; Hansen et al., 1989). However, electrophysiological findings in a retrospective study of cisplatin treated patients with testicular cancer (Krarup-Hansen et al., 1993), indicated that axonal degeneration was not predominant in the distal part of sensory fibres. Accumulation of cisplatin in the dorsal root ganglion, compared with peripheral and central nervous tissue, and loss of large neuronal cells (Thompson et al., 1984; Gregg et al., 1992; Krarup-Hansen et al., 1999) is consistent with a sensory neuronopathy (Sghirlanzoni et al., 2005). In connection with its pathological effect on dorsal root ganglion cells in experimental animals (Cavaletti et al., 1992; Cece et al., 1995), cisplatin has been proposed to interfere with protein synthesis and cause axonal dysfunction (Tomiya et al., 1986), or a dual action on DNA and axonal microtubules may cause distal axonal degeneration (Windebank et al., 1994). In analogy, the neuropathy caused by pyridoxine toxicity is due to a sensory neuronopathy (Schaumburg et al., 1983; Windebank et al., 1985), whereas the earliest abnormalities in an experimental study were associated with a distal axonopathy (Xu et al., 1989), raising the possibility of a primary distal effect of the interference with neuronal cell body metabolism. A distal ‘dying-back’ axonopathy may be reversible (Asbury, 1987), and sensory symptoms during treatment have been reported to resolve in many patients after discontinuation of cisplatin therapy (Hartmann et al., 1999). It remains possible that the earliest manifestations of the neuropathy are localized to the distal segment of sensory axons.

To assess whether the distal segments of sensory axons were disproportionately affected by cisplatin at early stages we followed pathophysiological changes at the most distal and at more proximal parts of sensory nerves. Three findings in our study indicated that the neurotoxic effect of cisplatin involves large myelinated sensory fibres throughout their course.

First, reduction of SNAP amplitudes developed in parallel in the fingers and the feet and at the same rate and to the same extent at the distal and proximal fingers and at the feet and the ankles. Thus at cumulative doses up to 300 mg/m² there was no convincing evidence of sensory fibre loss in the hands or the feet, whereas amplitudes of the compound sensory action potentials in both the median and the sural nerve decreased by 50–60% at the cumulative doses of 385–711 mg/m². The sensory conduction velocities along the distal and more proximal segments of the median and sural nerves decreased to the same extent by 10–15% also supporting that loss of large fibres was similar at the distal and more proximal nerve segments. In a dying back axonopathy a predominant effect would have been expected to take place in the feet before the hands and to be more pronounced at distal than at proximal sites of the fingers or the feet.

Secondly, the loss of both ankle jerks and H-reflexes at doses >300 mg/m² indicates that disruption of the monosynaptic reflex arch could not be explained by degeneration of the stretch receptors at the muscle spindle, but rather suggests loss of afferent input through loss of large sensory neurons. In contrast, the distal ‘dying-back’ acrylamide axonopathy in experimental animals was associated with loss of receptor triggered nerve action potentials whereas electrically evoked responses at more proximal sites were maintained (Sumner and Asbury, 1975; Sumner, 1980). In comparison, the F-waves to the soleus muscle remained normal indicating that the loss of the monosynaptic reflex was not due to reduced excitability of the motoneurons or to proximal nerve or root damage. The greater incidence of loss of ankle jerks than knee jerks at the same dose of cisplatin further indicates that the sensory nerve fibre loss was length dependent.

Thirdly, abnormalities in tactile responses evoked at digit 3 and the foot did not develop earlier or to a greater degree than loss of SNAPs evoked by electrical stimulation as would have been expected in a distal axonal degeneration. Tactile responses evoked by an indentation velocity of 400 μm/ms originate predominantly from Pacinian
corpuscles, innervated by the fastest conducting myelinated fibres, and to a lesser extent from more superficially located fast-adapting touch receptors that are innervated by slower conducting fibres (Simonetti et al., 1998; Baba et al., 2001). The amplitudes, receptor delays, latencies and conduction velocities before treatment with cisplatin were normal (Krarup-Hansen et al., 1993; Krarup and Trojaborg, 1994). The relationship between the increase in vibratory perception threshold and the reduction in the amplitude of the tactile response, which occurred during cisplatin treatment, was also consistent with its probable origin from Pacinian corpuscles (Mountcastle et al., 1972). It should be taken into account that the tactile SNAPs were less well defined than the SNAPs evoked by electrical stimulation due to the poorer synchronization of action potentials in different fibres and to the activation of fewer fibres by the tactile probe than the supramaximal electrical stimulus. The more dispersed shape and the lower amplitudes of the tactile response made it more difficult to delineate changes due to fibre loss. Nevertheless, the similarity between the reductions in conduction velocities of tactile and electrically evoked SNAPs, and the relationship between the percentage changes in amplitudes supported that the abnormalities in both types of responses occurred in parallel reflecting loss of large sensory neurons.

Whereas some studies have found autonomic disturbances after cisplatin treatment (Rosenfeld and Broder, 1984; Boogerd et al., 1990; Hansen, 1990; Quasthoff and Hartung, 2002), none of our patients had autonomic symptoms or signs, and the autonomic tests did not change during treatment. Temperature sensation was slightly but variably abnormal in four patients, and there was no effect on the longitudinal measurements during treatment.

The CNS involvement as indicated by the slowing of central conduction of the SEPs from the tibial nerve, confirming previous findings (Walsh et al., 1982; Dewar et al., 1986; Daugaard et al., 1987; Boogerd et al., 1990; Krarup-Hansen et al., 1993), occurred at the same doses of cisplatin as those that caused peripheral nerve changes. This similarity suggests that loss of large fibres in the dorsal columns was secondary to dorsal root ganglion cell degeneration.

Possible mechanism of cisplatin neurotoxicity

Our study proposes that the effect of cisplatin in moderate doses on the nervous system is localized to large cells of long fibres in the dorsal root ganglion, and this is consistent with morphometric measurements in post-mortem studies (Krarup-Hansen et al., 1999). This conclusion indicates selective susceptibility of large sensory neurons since temperature sensation conveyed by small sensory fibres, and autonomic neurons not protected by the blood–brain barrier, were not affected (Windebank, 1999). The binding of cisplatin to DNA and the formation of platinum–DNA adducts have been correlated with cytotoxicity (McDonald et al., 2005), and the induction of apoptosis has been investigated in sensory neurons in vitro and in vivo in experimental animals (Gill and Windebank, 1998; McDonald and Windebank, 2002). This question has, however, not been fully settled, and other mechanisms leading to cell death have been proposed (Gonzalez et al., 2001). Animal models of cisplatin neuropathy do not reproduce the changes seen in human patients; in particular axonal loss and degeneration occur only slightly or not at all (Tomiwa et al., 1986; Windebank et al., 1994).

In rodents, cisplatin at higher doses than usually used in humans in addition causes loss of small fibres but also has systemic effects (Bianchi et al., 2006), and it is not clear whether the results of these studies are relevant for toxicity in humans. It is not clear whether differences between human and animal susceptibility to the toxic effect of cisplatin may be related to species DNA repair ability. DNA repair has been suggested to be responsible for the resistance to apoptotic effects of cisplatin in certain tumour cell types (Jordan and Carmo-Fonseca, 2000; Kelland, 2000; Kartalou and Essigmann, 2001) and it may be speculated to influence species susceptibility to neurotoxicity.

Our findings support that the development of sensory neuropathy during cisplatin treatment includes degeneration of large dorsal root ganglion cell bodies with loss of large, long myelinated fibres both distally in the limbs and proximally in the spinal cord. The findings therefore have the implication that prevention of cisplatin neuropathy must rely on factors that inhibit the initiation of apoptosis or other mechanisms of neuronal degeneration (Alberts and Noel, 1995; Gao et al., 1995; Zheng et al., 1995; McDonald and Windebank, 2002).

Acknowledgements

The study was supported by the Lundbeck Foundation, the Novo Foundation, the Elsass Foundation, Danish Medical Research Council, Danish Masonic Order, F. C. Richter’s Foundation, E. W. Willumsen’s Foundation and The Danish Cancer Society. Funding to pay the Open Access Publication charges for this article was provided by the Department of Oncology, Righospitalet.

References

Cisplatin induced neuropathy


