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ORIGINAL ARTICLE

Interleukin-6 protects against paclitaxel, cisplatin and vincristine-induced neuropathies without impairing chemotherapeutic activity

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Abstract
Purpose This study was conducted to investigate the potential neuroprotective effect of IL-6 on chemotherapy induced neuropathy (CIN). IL-6 was compared to four-methylcatechol (4-MC)-a known inducer of NGF secretion previously shown to exhibit neuroprotective effects in CIN models.
Methods *Three CIN models were used;* two *in rats (cisplatin and vincristine) and* one *in mice (paclitaxel).* IL-6 was delivered in four different doses in rats (0.3, 1, 3, 10 μg/kg, sc) every day from the first day of chemotherapeutic agent intoxication until the end of the study (day 37 for cisplatin protocol and day 30 for vincristine procedure). In mice, IL-6 was delivered at 10 μg/kg, sc either daily or three times a week from the first day of intoxication until the end of the study (day 19). Behavioral testings (hot plate and rotarod), nerve conduction studies (CMAP, SNCV, H-wave) and histo-morphometric analysis were done for all models. In addition, we tested whether IL-6 interfered with the tumor-reducing effects of the chemotherapeutic agents.
Results IL-6 treatment prevented the behavioral and electrophysiological abnormalities produced by vincristine, cisplatin and Taxol intoxication, and similarly prevented the pathological changes in peripheral nerves. The neuroprotective action of chronic IL-6 treatment was at least equal to that of 4-MC. In addition, IL-6 neither inhibited the antitumour activity of cisplatin, nor stimulated tumour growth.
Conclusion IL-6 at low doses (10 μg/kg) provided protection against the development of CIN without demonstrating interference with the anti tumoural activity of these anti-mitotic drugs.

Keywords IL-6 · Chemotherapy-induced neuropathy · Neuroprotection

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Introduction

The neurotoxic side-effects of common chemotherapeutic agents, including platinum compounds, vinca alkaloids and taxanes, are the major reasons for stopping the anti-tumour therapy or changing the dose regimen. The chemotherapy-induced neuropathy (CIN) is partly reversible only and damages are definitively irreversible in the worst-case scenario. Thus, CIN represents an important and persistent limitation of quality of life even if drugs have successfully treated the cancer.

Vinca alkaloids act by binding on intracellular tubulin. Vincristine-driven neuropathy is mainly characterized by motor and sensory deficits. In the peripheral nervous system, the drug induces alterations in cellular microtubules leading to alterations of axonal transport and axonal degeneration. Many vincristine treated patients develop a dose-dependent (cumulative max. doses 30–50 mg), primarily sensory neuropathy [37, 45]. Early symptoms and signs of neuropathy are paraesthesia and pain of hands and feet, and distally accentuated hyperaesthesia. Attempts to prevent vincristine neuropathy using putative neuroprotective agents such as vitamin B_1, vitamin B_{12}, glutamate [9, 10], isoaxonine [36], insulin-like growth factor or nerve growth factor [3, 13, 22] have shown a very limited success.

Cisplatin (cis-diamine-dichloro-platinum) is an effective anti-tumour agent that is currently commonly used for the treatment of various malignancies and particularly ovarian, bladder, lung and testis cancer [43]. Other commonly used platinum derivatives are carboplatin and the more recently introduced oxaliplatin. The compounds act as anti-tumour agents by inducing intra- and interstrand crosslinks in DNA that result in apoptotic cell death by quickly dividing cell lines and cancer cells [28]. The platinum derivatives also have a high affinity for the peripheral nervous system [42]. These compounds can be detected in dorsal root ganglion cells (DRG) and in the sensory nerves themselves. The mechanism of toxicity is not due probably to direct damage of the DNA, but is based on a disturbance of cellular metabolism and axo-plasmatic transport.

Paclitaxel (Taxol) is an anticancer drug with a broad spectrum of activity against a wide variety of neoplasms, including breast, ovary, lung, and gastrointestinal tumors [48]. Peripheral neurotoxicity is the most restricting side-effect of Taxol [34]. More than half of the patients treated with Taxol >250 mg/m^2 describe parasthesias and dysaesthesia, which appear 24–72 h after administration. Some patients develop neuropathy even after a single administration of Taxol, especially in combination with cisplatin. In contrast to cisplatin, which predominantly affects the small and thinly myelinated axons (responsible for pain and temperature perception), Taxol preferentially affects large myelinated nerve fibres and consequently causes mixed sensory and motor dysfunction [24, 25].

Interleukin 6 (IL-6) has been proposed to act as a neurotrophic and neuroprotective factor and contribute to the maintenance of neuronal homeostatis [31, 54]. Experimental evidence has indicated that an increased IL-6 mRNA expression could be detected in acute degenerative processes (Wallerian degeneration) but not in chronic slower progressing neuropathies and not in intact nerve fibers [46]. In line with this, different neurotrophic factors including IL-6 were overexpressed in nerves after experimental axotomy. Also, they promoted axonal growth until axon/Schwann cell contact [14]. Moreover, it was shown that a chronic intraperitoneal as well as subcutaneous administration of IL-6 were protective against the development of diabetic-related neuropathy in streptozotoxin rats [2]. These findings implicate IL-6 in the neural degeneration and regeneration process.

The current study was undertaken in order to investigate the potential neuroprotective effect of IL-6 on vincristine and cisplatin-induced neuropathy in the rat and on Taxol-induced neuropathy in the mouse. Results shows that IL-6 treatment prevented the development of morphological changes induced by vincristine, cisplatin and Taxol intoxication and improved both behavioural, electrophysiological performance of animals. The neuroprotective action of chronic IL-6 treatment appeared more efficient than that of 4-methylcatechol (4-MC), a NGF inducer agent with neuroprotective property [16, 17], in different animal models of neurodegeneration [11, 49].

In addition, IL-6 neither inhibited the antitumour activity of these drugs, nor stimulated tumour growth. Therefore, IL-6 therapy may be an attractive approach to prevent the development of chemotherapy-induced neuropathies.

Materials and methods

Induction of cisplatin and vincristine neuropathy in rat and pharmacological treatment

Neuropathy was induced in 10-week-old female Dark Agouti rats by intraperitoneal (IP) injection of cisplatin (Sigma, France) twice a week at a dose of 2 mg/kg during 4 weeks (based on Boyle and co-workers [10]); or by a daily injection of vincristine (Tocris, France) from day 0 to 5, from day 8 to 12 and day 15 to 16 at a dose of 0.15 mg/kg (based on Boyle and co-workers [9]). The two drugs were diluted in 0.9% sterile aqueous solution of sodium chloride. IL-6 was diluted in saline containing 0.02% BSA and was administered at doses of 0.3, 1, 3, 10 μg/kg via subcutaneous (sc) route every day from the first day of chemotherapeutic agent intoxication (D1) until the end of the study (D37 for cisplatin and D30 for vincristine experiment). 4-MC, a known inducer of NGF secretion previously shown to exhibit neuroprotective effects, was diluted in saline and injected daily via IP route at the dose of 10 μg/kg, from the first day of chemotherapeutic agent intoxication until the end of the study. Six groups of ten rats were intoxicated with cisplatin or vincristine drugs and were treated with IL-6, 4MC or vehicle and an additional group of animals did not receive any intoxication and was treated with the vehicle (control group). The studies were initiated 1 week before the onset of cisplatin and

vincristine intoxication to acclimatize the animals to the handlings and tests and to allow measurement of baseline values. Body weight and survival rates were recorded on a daily basis and behavioural and electrophysiological monitorings were conducted weekly.

Induction of Taxol neuropathy in mouse and pharmacological treatment

Eight-week-old female C57Bl/6 mice were IP anesthetized with ketamine/xylazine mixure (4/1 v/v). Taxol (7.5 mg/ml in cremophor/ethanol, Sigma, USA) was injected into the jugular vein at a dose of 60 mg/kg every other day for 1 week (a total of three injections) (see [56] for further details). Control groups (0.02% BSA in saline) were injected with vehicle only. IL-6 was diluted in saline containing 0.02% BSA and was administered at doses of 10 μg/kg via sc route daily (7×) or three times a week (3×) from the first day of chemotherapeutic agent intoxication (D0) until the end of the study (D19). Five groups of ten animals were used. The studies were initiated one week before the onset of Taxol intoxication to acclimatize the animals to the handlings and tests and to allow measurement of baseline values. Body weight and survival rates were recorded on daily basis and behavioural and electrophysiological monitorings were conducted weekly. At the end of the study, all the animals were sacrificed.

For all experiences, behavioral tests were conducted on a weekly basis to train the animals and to detect the first impairments induced by chemotherapeutic agents. With this training and habituation procedure, all animals showed improvement of their performances throughout the study. This improvement was partially or fully hidden by the impairments induced by the chemotherapeutic treatments.

For all experiments, animals were housed two (rats) or five (mice) per cage and maintained in a room with controlled temperature (21–22°C) and a reversed light-dark cycle (12 h/12 h) with food and water available ad libitum. All experiments were carried out in accordance with institutional guidelines.

Behavioural measurements

Sensitivity test: hot plate test in rat

The rat was placed inside a glass cylinder of 17 cm height and 21 cm diameter on a heating-plate heated to 52°C (Medite OTS 40, Microm, France). The animal behavior was observed, particularly the licking of a foot and the adjusted leap. The latency before licking a foot or before jumping to escape the heat (adjusted leap) was recorded. The time needed to feel the thermal pain is related to the thermal sensitivity and tends to increase when thermal sensitivity is altered.

Motor-coordination test: Rotarod test in mouse

Motor-coordination was assessed using a Rotarod apparatus (Columbus Instruments, OH). Animals were acclimated to the protocol during the 3 days before each of the testing dates. Initial speed is 1.6 rpm, with acceleration rate of 4 rpm per minute. Animals were tested three times during each session with at least 2 min of rest between each test. The best performance for each testing date (before and after treatment) was recorded.

Nerve conduction studies in rats

Electrophysiological recordings (EMG) were performed using a Neuromatic 2000M electromyograph (Dantec, Les Ulis, France), the procedure was based on the work of Kennel and co-workers [18]. Rats were anaesthetized by IP injection of 60 mg/kg ketamine chlorhydrate (Imalgene 500®, Rhône Mérieux, France). The normal body temperature was maintained at 30°C with a heating lamp and verified using a contact thermometer (Quick, Bioblock Scientific, France) placed on the tail surface. H wave recording consisted in stimulating the sciatic nerve through a needle electrode (Dantec) at the sciatic notch level with a single 0.2 ms pulse at supramaximal intensity (12.8 mA) and measuring electromyographic responses from the plantar muscle. A monopolar recording electrode was inserted between the fourth and fifth digits, parallel to the long axis of the foot. The reference electrode was inserted into the fifth digit of the same foot, and a ground electrode was inserted into the tail. The first response, referred to as the M wave, results from direct stimulation of motor axons, whereas the second, or H wave, results from indirect stimulation of motoneurons mediated by sensory fibers. The amplitude and the latency of H wave signal were measured. Compound muscle action potential (CMAP) signal was recorded in the gastrocnemius muscle after stimulation of the sciatic nerve nerve through a needle electrode (Dantec) at the sciatic notch level. A reference electrode and an active needle were placed in the hindpaw. A ground needle was inserted on the lower back of the rat. Sciatic nerve was stimulated with a single 0.2 ms pulse at supramaximal intensity. The velocity of the motor wave was recorded and expressed in ms. Sensitive nerve conduction velocity (SNCV) was also recorded. The tail skin electrodes were placed as follows: a reference needle inserted at the base of the tail and an anode needle placed 30 mm away from the

reference needle towards the extremity of the tail. A ground needle electrode was inserted between the anode and reference needles. The caudal nerve was stimulated with a series of 20 pulses (for 0.2 ms) at an intensity of 12.8 mA. The signal velocity was expressed in m/s.

Nerve conduction studies in mice

Nerve conduction studies (Nicolet Viking Quest, Nicolet Biomedical, Madison, WI) were performed under methoxyflurane anesthesia. For hind limb recordings, electrodes were inserted into the interosseous muscles of the left foot; stimuli were administered transcutaneously at the ankle and at the hip (sciatic notch). Sensory nerve action potentials (SNAPs) were recorded in the tail. Subcutaneous recording electrodes were placed at the base of the tail, keeping the anode and the cathode $\sim$5 mm apart; stimuli were administered 4–5 cm distal. Waveforms were recorded from an average of 50 to 80 stimuli.

Morphometric analysis in rats

Twenty-three days post-cisplatin intoxication and 2 weeks after the end of vincristine intoxication three rats of each group were killed. Sciatic nerves, were excised, fixed overnight with 4% glutaraldehyde (Sigma, France) in PBS buffer (pH = 7.4) and maintained in 30% sucrose and stored at 4°C. At the time of use, the nerve sample was post-fixed in 1% osmium tetroxide (Sigma, France) in PBS for 2 h, dehydrated in serial alcohol solution, and embedded in Epon. Embedded tissues were then placed at 70°C during 3 days to allow polymerization of the epon resin. Cross sections of 1.5 μm thick were performed and they were stained with 1% toluidine blue solution (Sigma) for 2 min, dehydrated and mounted on Eukitt for light microscopy and image analysis using a computerized video image system. The morphometric analysis was based on Andriambeloson and co-workers procedure [2]. Briefly, six sections were examined using an optical microscope (Nikon, Tokyo, Japan) and analyzed using a semi-automated digital image analysis software (Biocom, France and Image Pro Inc., Houston, TX). Two randomly selected fields per slice were studied. The myelinated fibers with abnormal and normal appearances were analyzed. Myelinated fibers without axons, redundant myelin and fibers showing sheaths with thicknesses too large in respect to their axonal diameter were considered as abnormal fibers. Abnormal fibers were not taken into account from the myelin thickness and the fiber diameter measurements.

About 2,606 $\pm$ 78 fibers (including abnormal fibers) per animal were analysed.

Morphometric analysis (Mouse)

Two weeks after the last IL-6 injection, animals were perfused with 4% paraformaldehyde in 0.1 M PBS buffer (pH = 7.4). L4 dorsal and ventral roots were post-fixed overnight at 40°C in 5% glutaradehyde/0.1 M PBS, and embedded in plastic using standard techniques (see rat section). Cross sections were cut at 780 nM and stained with toluidine blue (1%) for light microscopy and image analysis using a computerized video image system. Digital images were captured and analyzed utilizing ImagePro software. The areas and mean diameters of all myelinated axons were measured by tracing the inner boarder of myelin sheath. Axonal density was calculated by dividing the number of axons by the area of nerve cross-section.

Mouse model of drug interaction

On D1, tumor growth was induced in Balb/c nude mice (Charles River Italia, S.p.a), by sc inoculation of $10^7 \pm 5\%$ WiDr colon adenocarcinoma cells (derived from a 78-year-old woman, accession number: ICLC HTL00003, National Cancer Institute, Genova, Italy). WiDr cells were selected for their responsiveness to IL-6 [53]. The animals were randomly allocated to experimental groups (n = 8) as following: (a) group sc administered with 0.02% mouse albumin (Sigma, Italy) in 40 mM phosphate buffer at pH = 7.0; (b) group ip administered with 3.5 mg/kg cisplatin (in 0.9% NaCl sterile solution for injection); (c) three groups treated with IL-6 (in 0.02% mouse albumin in 40 mM phosphate buffer at pH = 7.0), s.c. at 3, 10 or 30 μg/kg daily from D4 to D18 and (d) three groups of mice treated with IL-6 either at 3, 10 or 30 μg/kg IL-6, s.c. in combination with cisplatin from D4 to D18. Cisplatin (Sigma, Italy) was administered ip at 3.5 mg/kg on days 4, 8, 12, and 18. Tumor size was measured using a calliper twice a week for 4 weeks starting 4 days after cell inoculation.

Tumor volume (in mm^3) was estimated according to the following formula:

$$\text{Tumor volume}\left(\text{in mm}^3\right) = (\text{longer diameter}) \times (\text{shorter diameter})^2/2.$$

Data analysis

All data related to the neuropthic assays were analyzed by repeated analysis of variance (ANOVA). Dunnett's test as post-hoc analysis was used for multiple comparisons when appropriated. The level of significance was set at $P \leq 0.05$. The following symbol is used in the figures: # and

*$P < 0.05$. Results are expressed as mean ± standard error of the mean (SEM), $n = 8$–10 per group. One-way ANOVA followed by Tukey test were carried out for the mouse model of drug interaction.

Results

No statistical difference between all groups was observed in the baseline body weight, as well as EMG and behavioural recordings.

Cisplatin experiment

Animal body weight

Animals treated with cisplatin regardless of treatments undertaken demonstrated a significantly lower body weight compared to the control group ($P \leq 0.001$) (Fig. 1a). Once the cisplatin intoxication was withdrawn, the animals gained weight rapidly. No sign of general or local (at the site of injection) intolerance to IL-6 or 4-MC was noted.

Sensitivity test: hot plate

This test was used to measure the decrease of the mean time hot plate threshold induced by cisplatin after a long and massive intoxication as previously seen by other authors [4, 10].

On day 36, cisplatin-intoxicated groups treated with IL-6 showed an overall reduction of first reaction time when compared to the vehicle-treated group (Fig. 1b, $P \leq 0.05$ except 3 µg/kg). At the dose of 10 µg/kg, the effect of IL-6 treatment was obviously comparable to the one of the control. Similarly, 4-MC treatment produced a significant decrease of the first reaction time in cisplatin-intoxicated rats.

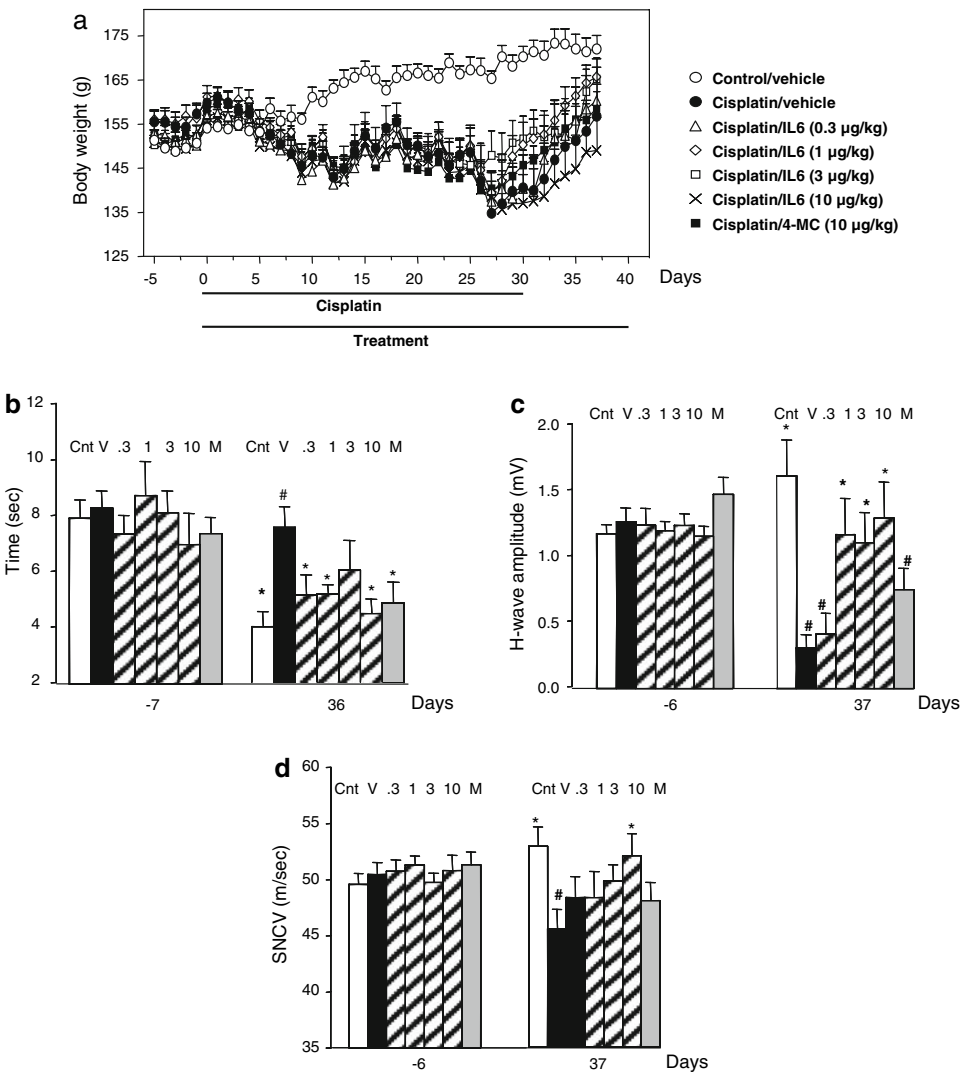

Fig. 1 Beneficial effects of IL-6 treatment in rats intoxicated with cisplatin. Body weight (**a**), sensitivity test (hot plate, **b**) electrophysiological recordings (SNCV, **c** and H-wave, **d**), in cisplatin intoxicated rats after daily SC administration of vehicle (*V, black bars*), or different doses of IL-6 (0.3, 1, 3 and 10 µg/kg, *hatched bars*) or 4-MC (M, *grey bars*) compared to non intoxicated rats receiving daily SC administration of the vehicle (C, *open bars*). *Cnt* nonintoxicated vehicle treated group; *V* cisplatin intoxicated vehicle treated group; 0.3, 1, 3 and 10 cisplatin intoxicated groups treated with the indicated concentration (µg/kg) of IL-6; *M* cisplatin intoxicated animals treated with 4-MC. Data are expressed as mean ± standard error of the mean (SEM), $n = 8$–10 per group. *$P < 0.05$ versus cisplatin/vehicle (V) group; #$P < 0.05$ versus control/vehicle (Cont) group

Electrophysiological measurements

Amplitude of H wave

The amplitude of H wave was recorded (Fig. 1c), the animals showing no wave after the stimulation were credited with 0 mV amplitude. The percentage of zero per group was recorded and given as informative data in Table 1.

The change in the H wave amplitude in the vehicle-treated (Fig. 1c) was dramatically reduced by about 80% when compared to the control group. Whilst IL-6 used at the dose of 0.3 μg/kg appeared to have no effect, highest doses (1–10 μg/kg) were found to significantly prevent the loss of H wave. For example, at 1–10 μg/kg doses, the wave amplitude was comparable to the control one ($P > 0.05$ vs. control/vehicle group). 4-MC treatment reduced (not significantly) the loss of H wave induced by cisplatin. The proportion of animals demonstrating H wave was markedly reduced in vehicle-treated cisplatin intoxicated rats during the last 3 weeks of the study (day 22, 29, 37, Table 1). When treated with either IL-6 or 4-MC, the percentage of animals lacking H wave on day 37 was markedly reduced (about 10%), except in the group receiving the lowest dose of IL-6 where about 40% of animals did not demonstrate H wave.

Table 1 Percentage of animals demonstrating H wave

Group	Day 7	Day 22	Day 29	Day 36
Control/vehicle	100	100	100	100
Cisplatin/vehicle	100	70	22.22	75
Cisplatin/IL-6 (0.3 μg/kg)	100	66.67	66.67	55.56
Cisplatin/IL-6 (1 μg/kg)	100	100	80	90
Cisplatin/IL-6 (3 μg/kg)	100	100	90	100
Cisplatin/IL-6 (10 μg/kg)	100	100	90	90
Cisplatin/4-MC (10 μg/kg)	100	100	90	80

Percentage of rats intoxicated with vehicle or cisplatin demonstrating H-wave from D 7 (baseline) to D 36 (end of the study) after IL-6 or 4-MC treatment. n = 8–10 per group

Sensory nerve conduction velocity SNCV

The first alterations of SNCV parameter induced by cisplatin intoxication were observed at day 23 (data not shown). On D37, the signal velocity was significantly lower in vehicle treated animals (Fig. 1d) than in control animals. The treatment with IL-6 (0.3, 1, 3, 10 μg/kg) improved the nerve conduction velocity in cisplatin-intoxicated animals, but only the dose of 10 μg/kg reached the significance. 4-MC treatment appeared to slightly prevent (but not significantly) the decrease in SNCV of cisplatin-intoxicated rats.

Morphometric analysis

The sciatic nerves harvested at day 23 pi from intoxicated rats (Table 2) showed a significant decrease in the fiber diameter and myelin thickness and an increase in the percentage of abnormal nerve fibers when compared to controls. IL-6 treatment at the dose range of 1–10 μg/kg prevented cisplatin-induced nerve degeneration, while the lower dose (0.3 μg/kg) showed no effect. As expected, 4-MC treatment protected against cisplatin-induced fiber degeneration. IL-6 (1–10 μg/kg) and 4-MC treatments were also found to significantly prevent the cisplatin-induced decrease fiber diameter and myelin sheath thickness.

Table 2 Morphometric analysis in cisplatin intoxicated rats (fiber diameter, myelin thickness and abnormal fibers)

Parameters	Control/vehicle	Cisplatin/ vehicle	Cisplatin/IL-6 (0.3 μg/kg)	Cisplatin/IL-6 (1 μg/kg)	Cisplatin/IL-6 (3 μg/kg)	Cisplatin/IL-6 (10 μg/kg)	Cisplatin/4-MC (10 μg/kg)
Fiber diameter (μm)	11.207 ± 0.303*	8.970 ± 0.165	9.448 ± 0.272	10.251 ± 0.181*	10.048 ± 0.176*	10.391 ± 0.194*	10.207 ± 0.189*
Myelin thickness (μm)	2.659 ± 0.073*	2.111 ± 0.038	2.245 ± 0.066	2.415 ± 0.044*	2.358 ± 0.040*	2.467 ± 0.047*	2.424 ± 0.046*
Abnormal fibers (%)	17.55 ± 1.07*	31.19 ± 3.06	31.10 ± 1.99	25.42 ± 1.31*	23.32 ± 1.66*	22.74 ± 1.10*	20.02 ± 1.19*

Morphometric analysis in cisplatin intoxicated rats after daily SC administration of vehicle, or different doses of IL-6 (0.3, 1, 3 and 10 μg/kg) or 4-MC (10 μg/kg) compared to nonintoxicated rats receiving daily SC administration of the vehicle (control/vehicle). Data are expressed as mean ± standard error of the mean (SEM), n = 8–10 per group

* $P < 0.05$ versus Cisplatin/vehicle group

Vincristine experiment

Animal body weight

Vincristine intoxication induced a marked inhibition of animal growth (10% of the body weight). IL-6 and 4-MC were found to be ineffective on the vincristine-induced body weight loss. Once the vincristine was withdrawn, regardless of treatments undertaken, animals gained weight rapidly (Fig. 2a).

Sensory function: hot plate test

This test was used to measure the decrease of the mean time hot plate threshold induced by vincristine after a massive intoxication as previously seen by other authors [9, 13].

Hot plate test results (Fig. 2b) showed that vincristine intoxication significantly delayed the first reaction to heat in vincristine/vehicle-treated group when compared to control group. IL-6 treatment (0.3–3 μg/kg, on week 5) significantly decreased the time to the first reaction; Il-6 at the highest dose (10 μg/kg) delayed the first reaction without reaching the significance. 4-MC treatment did not appear to reverse the reaction to the heat impairments induced by vincristine.

Nerve conduction studies

Amplitude of compound muscular action potential A significant reduction in the CMAP amplitude was observed from 3 weeks in vehicle-treated group (data not shown), where animals lost about 20% of amplitude when compared to the control. Similar extent of loss was also observed until the study termination (week 5), although vincristine intoxication was no longer present (Fig. 2c). When used at the dose of 0.3 μg/kg, IL-6 appeared to having no effect, higher doses (1, 3 and 10 μg/kg) in contrast induced full protection of CMAP amplitude. Similarly, 4-MC significantly prevented vincristine-induced CMAP amplitude loss.

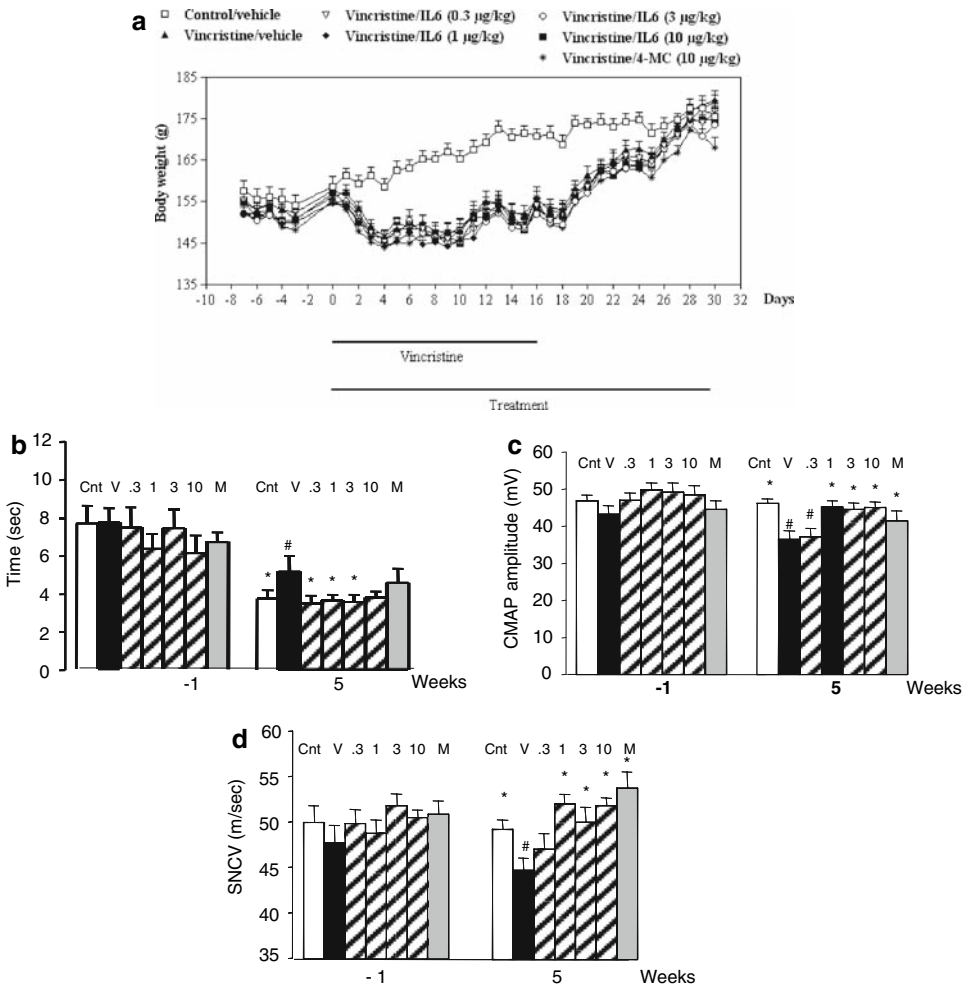

Fig. 2 Beneficial effects of IL-6 treatment in rats intoxicated with vincristine. Body weight (**a**), sensitivity test (hot plate, **b**) electrophysiological recordings (SNCV, **c** and H-wave, **d**), in vincristine intoxicated rats after daily SC administration of vehicle (V, *black bars*), or different doses of IL-6 (0.3, 1, 3 and 10 μg/kg, *hatched bars*) or 4-MC (M, *grey bars*) compared to nonintoxicated rats receiving daily SC administration of the vehicle (C, *open bars*). *Cnt* nonintoxicated vehicle treated group; *V* vincristine intoxicated vehicle treated group; 0.3, 1, 3 and 10 vincristine intoxicated groups treated with the indicated concentration (μg/kg) of IL-6; *M* vincristine intoxicated animals treated with 4-MC. Data are expressed as mean ± standard error of the mean (SEM), $n = 8$–10 per group. $^{*}P < 0.05$ versus cisplatin/vehicle (V) group; $\#P < 0.05$ versus control/vehicle (Cont) group

Sensory nerve conduction velocity The first change in SNCV occurred at the end of intoxication period (week 2) in vehicle-treated rats (data not shown). During the post-intoxication period, the alteration became substantial with a loss of about 7 m/s on week 5 when compared to the value obtained from control animals (Fig. 2d). The effect of IL-6 treatment was mainly detected toward the end of the study as a full restoration of SNCV could be observed at the highest doses (except at 0.3 μg/kg) on week 5. Similar significant result was obtained with 4-MC as the treatment promoted a full recovery.

Morphometric analysis

The morphological analysis showed that the percentage of abnormal fibers in samples collected from vincristine intoxicated rats (Table 3) were two times higher than in samples from control rats. When treated with either IL-6 (all doses) or 4-MC, the percentage of abnormal fibres was significantly reduced. Moreover, the myelin thickness and the fiber diameter were significantly reduced after vincristine intoxication, IL-6 (1–10 μg/kg) and 4-MC treatments were also found to significantly prevent these impairments.

Paclitaxel experiment

No difference in the body weight between Taxol and vehicle treated mice was observed (data not shown).

Motor-coordination function: rotarod test

Rotarod testing performed on D19, showed a significant difference between control group and the mice intoxicated with Taxol (Fig. 3a). IL-6 treated mice (for the two schedules of injection) showed a slight but not significant increase in the rotarod performances. By contrast, 4-MC showed a significant reduction of the rotarod impairment induced by Taxol treatment.

Sensory nerve action potential amplitudes

A significant decrease of the SNAP was recorded on D19 (15 days pi), IL-6 showed significant protection of SNAP, but only the 3× schedule reached a significant protection. Similar pattern of result with IL-6 (3×) was obtained with 4-MC (Fig. 3b).

Morphometric analysis

The morphological analysis performed 16 days pi showed that the axonal density fibers was two times higher in samples from control mice than in Taxol intoxicated samples (Fig. 3c). When treated with either IL-6 (10 μg/kg 3×) or 4-MC, the percentage of abnormal fibers was significantly reduced.

Drug interaction in vivo

This experiment was carried out to investigate the potential drug interaction between IL-6 and the chemotherapeutic agent in vivo. Cisplatin administered at 3.5 mg/kg at days 4, 8, 12, 18 (Fig. 4a) was able to reduce tumor growth and the antiproliferative effect was maintained also after interruption of treatments. Significant inhibitions versus vehicle control group were achieved at days 18 ($P < 0.05$), 22 ($P < 0.01$) and 25 ($P < 0.001$). IL-6 alone did not significantly affect tumor growth (Fig. 4a).

When IL6 was combined with cisplatin (Fig. 4b), a marked reduction of tumor growth was observed. Only the

Table 3 Morphometric analysis in vincristine intoxicated rats (fiber diameter, myelin thickness and abnormal fibers)

Parameters	Control/vehicle	Vincristine/vehicle	Vincristine/IL-6 (0.3 μg/kg)	Vincristine/IL-6 (1 μg/kg)	Vincristine/IL-6 (3 μg/kg)	Vincristine/IL-6 (10 μg/kg)	Vincristine/4-MC (10 μg/kg)
Fiber diameter (μm)	9.051 ± 0.154*	8.027 ± 0.141	7.640 ± 0.134	8.773 ± 0.142*	8.550 ± 0.142	8.892 ± 0.178*	8.683 ± 0.183*
Myelin thickness (μM)	2.159 ± 0.037*	1.894 ± 0.033	1.817 ± 0.032	2.077 ± 0.035*	2.024 ± 0.035*	2.116 ± 0.042*	2.062 ± 0.045*
Abnormal fibers (%)	19.85 ± 1.06*	41.37 ± 2.16	36.04 ± 1.70*	23.60 ± 1.11*	22.49 ± 1.20*	22.32 ± 1.09*	23.92 ± 1.54*

Morphometric analysis in vincristine intoxicated rats after daily SC administration of vehicle, or different doses of IL-6 (0.3, 1, 3 and 10 μg/kg) or 4-MC (10 μg/kg) compared to nonintoxicated rats receiving daily SC administration of the vehicle (control/vehicle). Data are expressed as mean ± standard error of the mean (SEM), $n = 8$–10 per group

* $P < 0.05$ versus Vincristine/vehicle group

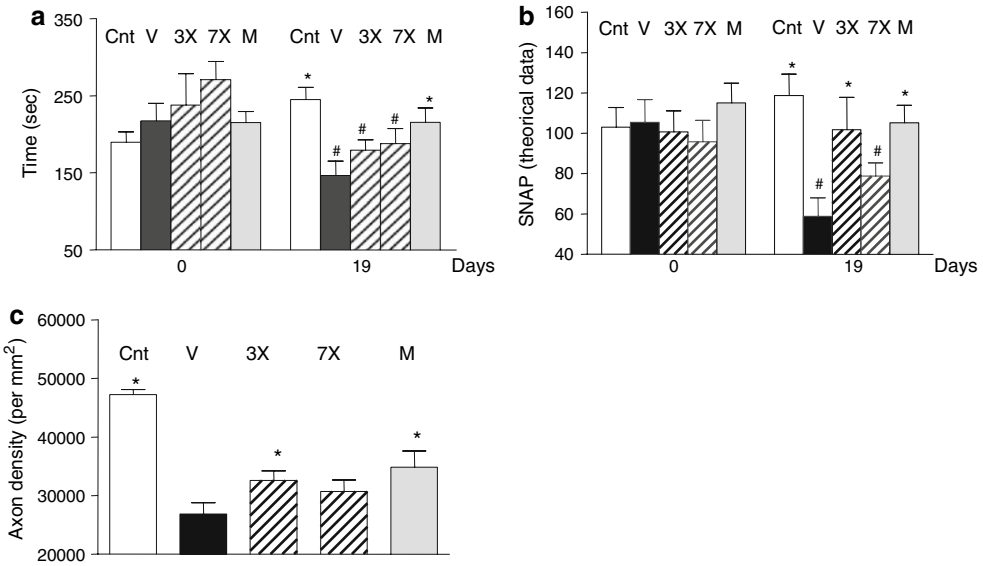

Fig. 3 Beneficial effects of IL-6 treatment in mice intoxicated with Taxol. Motor-coordination test (rotarod, **a**), electrophysiological recording (SNAP, **b**) and morphometric analysis (**c**) in Taxol intoxicated mice after daily sc administration of vehicle (V, *black bars*), different timing of IL-6 (10 μg/kg, 3X: 3 times a week, 7X: daily, *hatched bars*) or 4-MC (M, *grey bars*) in Taxol intoxicated mice compared to non intoxicated mice receiving daily sc administration of the vehicle (C, *open bars*). *C* non intoxicated vehicle treated group; *V* Taxol intoxicated vehicle treated group; 3X and 7X Taxol intoxicated groups treated with 10 μg/kg of IL-6 3 times a week or daily respectively; *M* Taxol intoxicated animals treated with 4-MC. Data are expressed as mean ± standard error of the mean (SEM), $n = 8$ per group. *$P < 0.05$ versus cisplatin/vehicle (V) group; #$P < 0.05$ versus control/vehicle (Cont) group

combination of the two higher doses of IL-6 with cisplatin resulted in a significant inhibition of tumor growth ($P < 0.01$ day 22 both 10 and 30 mcg/kg; $P < 0.05$ and 0.01 day 25 for 10 and 30 mcg/kg, respectively). However, no significant difference between all the combination treated groups and cisplatin-treated group was found.

Discussion

Current therapy for CIN is restricted to symptomatic treatments of paraesthesia and pain. There are no drugs on the market to prevent CIN, even though this disorder can be predicted based on the patient population receiving chemotherapeutic drugs. Neurotrophic factors seem to provide with an attractive approach to prevent and to cure CIN [3]. In CIN animal models, NGF and IGF were able to reduce or even prevent neuropathy [4, 13, 22, 38]. Furthermore, some authors have reported potent analgesic effects of glia derived neurotrophic factors (GDNF) in neuropathic pain suggesting this compound as future treatment of CIN [8]. Unfortunately these promising substances have not reached clinical application so far. One of the reasons is based on difficulties with drug administration, adverse effects and pharmacokinetics. Furthermore, until now it is still unclear how nerve growth factors might effect tumour proliferation. Other neuroprotective drugs that are used in diabetic neuropathy might be also helpful in CIN, e.g. alpha-lipoic acid [59]. Attempts to prevent CIN by the calcium channel blocker nimodipine have failed [12]. Glutamate ameliorates the behavioural manifestations experimental vincristine, cisplatin and Taxol neuropathies [9, 10]. Calpain inihibition has also shown promise for prevention of CIN in an animal model of Taxol neuropathy [55].

Interleukin-6 (IL-6) belongs to a cytokine family which includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), interleukin-11 (IL-11), cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC) (also known as novel neurotrophin-1/B cell stimulating factor-3) [15, 51]. Since these cytokines regulate proliferation and differentiation both in the haematopoietic and neuronal systems, they also have been termed haematopoietic/neuropoietic cytokines [5]. A variety of in vitro and in vivo studies demonstrated the neuroprotective actions of IL-6, such as the effect on basal cortical and mesencephalic or enteric neuron survival [21, 35, 50], and on sensory neurons [19]. In addition, pretreatment with IL-6 of cultured hippocampal neurons protects against glutamate or NMDA toxicity [27, 52, 56]. Similar neuroprotective effects are observed on cultured granule neurons after glutamate intoxication [44]. Moreover, in vitro IL-6 protects dopaminergic neurons against MPP+ induced toxicity [1]. In vivo, IL-6 shows neuroprotection on different models of neurodegeneration involving different population of neurons such as axotomy

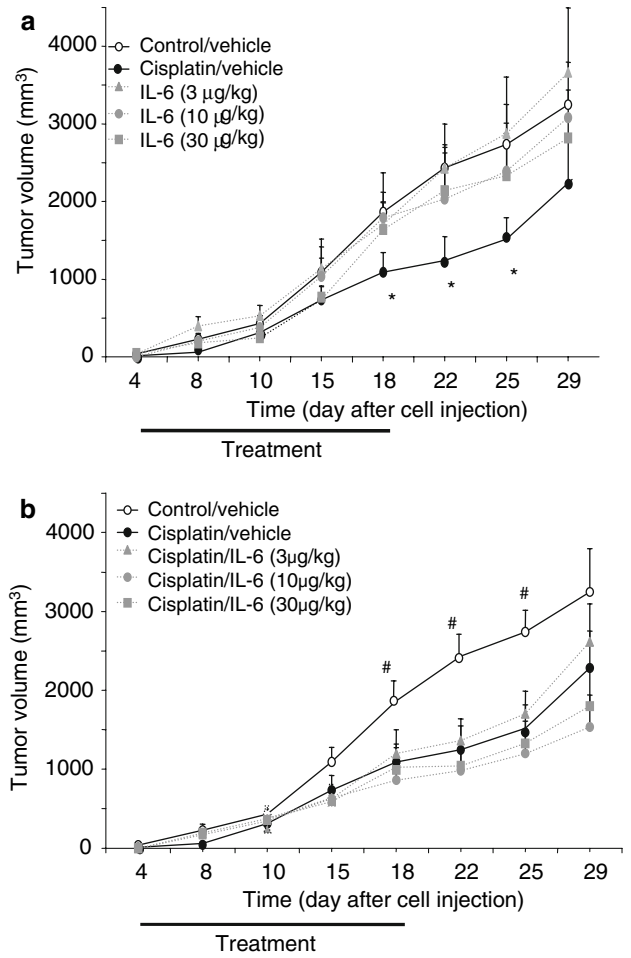

Fig. 4 Effect of IL-6 on cisplatin induced anti-tumor activity in vivo. Tumor size recorded twice a week for 4 weeks starting 4 days after cell inoculation. Tumor size in animals treated with vehicle, cisplatin and IL-6 alone (**a**). Tumor size in animals treated with vehicle, cisplatin alone and cisplatin/IL-6 combination (**b**). Data are expressed as mean ± standard error of the mean (SEM), $n = 8$–10 per group. $^{*}P < 0.05$ versus control/vehicle group; $\#P < 0.05$ versus cisplatin/vehicle group

[29], ischemia [41], nerve injury [26]. In the wobbler mice, a motor neuron degeneration model IL-6 delays the progression of the disease [30]. The quinolinic acid rat model of Huntington's syndrome shows a neuroprotective effect [6] as well as in a rat model of diabetic induced neuropathy [2].

The current study was undertaken in order to investigate the potential neuroprotective effect of IL-6 on cisplatin, vincristine and Taxol-induced neuropathies.

In a previous study, Boyle and co-workers [10] have developed a rat model of cisplatin-induced neuropathy, where sensory peripheral impairment (significant elevations of mean tail-flick threshold) has been reported. Using the similar animal model and behavioural tests, we assessed the therapeutic effect of IL-6. Moreover, electrophysiological and morphological correlates were also undertaken in order to unequivocally establish the effectiveness of IL-6 therapy. Results showed that IL-6 treatment prevented the development of morphological changes (abnormal fibers) induced by cisplatin intoxication as well as the fiber atrophy (fiber diameter, myelin thickness) occurring in this model [24]. As a result, the electrophysiological parameters (H wave amplitude and SNCV) of cisplatin intoxicated rats have been improved and became comparable with those treated with 4-MC, a standard compound with an established neuroprotective action [2, 11]. Importantly, the above results were correlated with improved sensory behavioural parameters (reaction to heat). It could be pointed out that the most efficient IL-6 dose was of 10 μg/kg. The lowest dose showed efficacy but not constantly reach the significance.

Again, Boyle and co-workers [9] have developed a rat model of vincristine-induced neuropathy, where sensory and motor peripheral defects as measured by behavioural tests (hot plate and walking test, respectively) have been reported to closely resemble the condition in human than any previously described. Using the same animal model and similar conventional behavioural tests as above-implemented by Boyle, we wished to assess the therapeutic activity of IL-6 with regard to the treatment of vincristine-induced neuropathy. Here, we found that IL-6 therapy improved both sensory and motor defects of vincristine intoxicated animals. A large preservation of fiber function was observed after IL-6 treatment as indicated by normal-appearing CMAP and SNCV, especially when used at the highest dose (1–10 μg/kg). Also, histomorphometric analyses performed at the completion of the study provided a clear result of the protective effect of IL-6 therapy on the fiber atrophy, as well as on the degeneration process (abnormal fiber parameter).

In this model by contrast with cisplatin model, all tested IL-6 doses even low doses were efficient in all parameters.

Moreover in mice intoxicated with Taxol, we proved that IL-6 (10 μg/kg given three times a week) was able to protect the electrophysiological and morphological damages (fiber density) induced by Taxol. Again in this model, the neuroprotective effect of IL-6 was similar to the 4MC reference molecule.

Altogether, these findings strongly suggest that IL-6 could be an additive therapy to prevent the development of neuropathy caused by vincristine, cisplatin and Taxol.

Furthermore, on a model of colon cancer treated with cisplatin, we clearly proved that the neuroprotective effect of IL-6 did not interfere with the antitumoral activity of cisplatin. No inhibition or decrease of the cisplatin anti-proliferation efficacy was observed. We did other tumoral cell vitro models treated by anti-mitotic agents (vincristine, cisplatin as well as Taxol) to highlight any interaction with IL-6. No inhibition or decrease of the chemotherapeutic agents efficacy was observed (data not shown).

In various rodent models of neurodegeneration (CIN in the current study as well as rat model of diabetic neuropathy [2]) the beneficial effect of IL-6 was observed at very low doses (10 μg/kg). This range of efficacy was lower than those used in other disease models in which IL-6 was active. e.g. thrombopoiesis, in these models the IL-6 efficient dose was found around 500 μg/kg [23]. These differences in concentration range could be explained by a different mode of action of IL-6 neuroprotective action.

The underlying mechanism(s) of IL-6 in this model remains to be established. Although this issue was not specifically addressed in the present study, it has been proposed that IL-6 promotes nerve regeneration processes. The neuroprotective effect of IL-6 demonstrated in the present study is in accordance with previously documented effects of IL-6 on sensory and motor neurons. Marz and coworkers [40] have shown that IL-6 in conjunction with the IL-6R can confer IL-6 sensitivity to sympathetic neurons, resulting in enhanced neuronal survival in the absence of NGF and induction of neuropeptides and choline acetyltransferase. Moreover, IL-6 is rapidly elevated following axotomy of peripheral nerves [7, 33]. Hirota and coworkers [26] have identified on injured neurons and on glial cells, cellular sites of IL-6 production; they showed IL-6 local accumulation following a nerve injury accelerating functional nerve regeneration. In vivo, IL-6 injections appeared to reduce demyelination in the murine encephalomyelitis model produced by Theiler's virus infection [47]. The IL-6 cytokine family, including CNTF and LIF, has major biological roles by signalling through a common receptor subunit, glycoprotein (gp) 130. The role of the activation of the gp130 signalling pathway is essential in the process of nerve regeneration and/or survival, as suggested by the potentiation of the neuroprotective effects of IL-6 following co-administration of its soluble receptor [32]. This concept is further supported by the fact that mutant mice lacking a functional LIF-R to trigger the activation of gp130 shows a loss of more than 35% of facial motor neurons, 40% of spinal motor neurons and 50% of neurons in the nucleus ambiguous [39]. In addition, gp130 is required for the integrity of the myelin sheath as conditional gp130-mutant mice show a progressive alteration of the myelin sheath structure [58]. Activation of gp130 signalling by IL6R/IL6, an interleukin-6 receptor-interleukin-6 fused molecule appears comparable to cyclic AMP elevating agents such as forskolin, known to induce the myelin gene products (myelin basic protein and myelin protein zero genes) in DRG and Schwann cell cultures [20]. IL-6 could act in our CIN models enhancing regeneration via gp130 signalling by IL6R/IL6. However, because the present study did not specifically address the mechanism(s) by which IL-6 protects against the development of CIN neuropathy or repairs the damage induced by anti-mitotic agents, additional investigations should be carried out in order to confirm and further characterize modulation of gp130 signalling pathway by IL-6 in the present models.

In conclusion, IL-6 at low doses (10 μg/kg) protects against CIN. IL-6 is promising as a potential treatment for prevention of induced neuropathy in patients treated with neurotoxic chemotherapies.

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References

1. Akaneya Y, Takahashi M, Hatanaka H (1995) Interleukin-1 beta enhances survival and interleukin-6 protects against MPP+ neurotoxicity in cultures of fetal rat dopaminergic neurons. Exp Neurol 136:44–52
2. Andriambeloson E, Baillet C, Vitte PA, Garotta G, Dreano M, Callizot N (2006) Interleukin-6 attenuates the development of experimental diabetes-related neuropathy. Neuropathology 26:32–42
3. Apfel SC (1996) Neurotrophic factors in the treatment of neurotoxicity: an overview. Neurotoxicology 17:839–844
4. Apfel SC, Arezzo JC, Lipson L, Kessler JA (1992) Nerve growth factor prevents experimental cisplatin neuropathy. Ann Neurol 31:76–80
5. Bazan JF (1991) Neuropoietic cytokines in the hematopoietic fold. Neuron 7:197–208
6. Bensadoun JC, de Almeida LP, Dreano M, Aebischer P, Deglon N (2001) Neuroprotective effect of interleukin-6 and IL6/IL6R chimera in the quinolinic acid rat model of Huntington's syndrome. Eur J Neurosci 14:1753–1761
7. Bolin LM, Verity AN, Silver JE, Shooter EM, Abrams JS (1995) Interleukin-6 production by Schwann cells and induction in sciatic nerve injury. J Neurochem 64:850–858
8. Boucher TJ, Okuse K, Bennett DL, Munson JB, Wood JN, McMahon SB (2000) Potent analgesic effects of GDNF in neuropathic pain states. Science 290:124–127
9. Boyle FM, Wheeler HR, Shenfield GM (1996) Glutamate ameliorates experimental vincristine neuropathy. J Pharmacol Exp Ther 279:410–415
10. Boyle FM, Wheeler HR, Shenfield GM (1999) Amelioration of experimental cisplatin and paclitaxel neuropathy with glutamate. J Neurooncol 41:107–116
11. Callizot N, Warter JM, Poindron P (2001) Pyridoxine-induced neuropathy in rats: a sensory neuropathy that responds to 4-methylcatechol. Neurobiol Dis 8:626–635
12. Cassidy J, Paul J, Soukop M, Habeshaw T, Reed NS, Parkin D, Kaye SB (1998) Clinical trials of nimodipine as a potential neuroprotector in ovarian cancer patients treated with cisplatin. Cancer Chemother Pharmacol 41:161–166
13. Contreras PC, Vaught JL, Gruner JA, Brosnan C, Steffler C, Arezzo JC, Lewis ME, Kessler JA, Apfel SC (1997) Insulin-like growth factor-I prevents development of a vincristine neuropathy in mice. Brain Res 774:20–26
14. Creange A, Barlovatz-Meimon G, Gherardi RK (1997) Cytokines and peripheral nerve disorders. Eur Cytokine Netw 8:145–151
15. Elson GC, Lelievre E, Guillet C, Chevalier S, Plun-Favreau H, Froger J, Suard I, de Coignac AB, Delneste Y, Bonnefoy JY,

Gauchat JF, Gascan H (2000) CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex. Nat Neurosci 3:867–872
16. Furukawa Y, Fukazawa N, Miyama Y, Hayashi K, Furukawa S (1990) Stimulatory effect of 4-alkylcatechols and their diacetylated derivatives on the synthesis of nerve growth factor. Biochem Pharmacol 40:2337–2342
17. Furukawa Y, Tomioka N, Sato W, Satoyoshi E, Hayashi K, Furukawa S (1989) Catecholamines increase nerve growth factor mRNA content in both mouse astroglial cells and fibroblast cells. FEBS Lett 247:463–467
18. Kennel P, Fonteneau P, Martin E, Schmidt JM, Azzouz M, Borg J, Guenet JL, Schmalbruch H, Warter JM, Poindron P (1996) Electromyographical and motor performance study in the *pmn* model of neurodegenerative disease. Neurobiol Dis 3:137–147
19. Gadient RA, Otten U (1996) Postnatal expression of interleukin-6 (IL-6) and IL-6 receptor (IL-6R) mRNAs in rat sympathetic and sensory ganglia Brain Res 724:41–46
20. Haggiag S, Chebath J, Revel M (1999) Induction of myelin gene expression in Schwann cell cultures by an interleukin-6 receptor-interleukin-6 chimera. FEBS Lett 457:200–204
21. Hama T, Miyamoto M, Tsukui H, Nishio C, Hatanaka H (1989) Interleukin-6 as a neurotrophic factor for promoting the survival of cultured basal forebrain cholinergic neurons from postnatal rats. Neurosci Lett 104:340–344
22. Hayakawa K, Itoh T, Niwa H, Mutoh T, Sobue G (1998) NGF prevention of neurotoxicity induced by cisplatin, vincristine and Taxol depends on toxicity of each drug and NGF treatment schedule: in vitro study of adult rat sympathetic ganglion explants. Brain Res 794:313–319
23. Herodin F, Mestries JC, Janodet D, Martin S, Mathieu J, Gascon MP, Pernin MO, Ythier A (1992) Recombinant glycosylated human interleukin-6 accelerates peripheral blood platelet count recovery in radiation-induced bone marrow depression in baboons. Blood 80:688–695
24. Hilkens PH, Pronk LC, Verweij J, Vecht CJ, van Putten WL, van den Bent MJ (1997) Peripheral neuropathy induced by combination chemotherapy of docetaxel and cisplatin. Br J Cancer 75:417–422
25. Hilkens PH, ven den Bent MJ (1997) Chemotherapy-induced peripheral neuropathy. J Peripher Nerv Syst 2:350–361
26. Hirota H, Kiyama H, Kishimoto T, Taga T (1996) Accelerated Nerve Regeneration in Mice by upregulated expression of interleukin (IL) 6 and IL-6 receptor after trauma. J Exp Med 183:2627–2634
27. Holliday J, Parsons K, Curry J, Lee SY, Gruol DL (1995) Cerebellar granule neurons develop elevated calcium responses when treated with interleukin-6 in culture. Brain Res 673:141–148
28. Huang H, Zhu L, Reid BR, Drobny GP, Hopkins PB (1995) Solution structure of a cisplatin-induced DNA interstrand cross-link. Science 270:1842–1845
29. Ikeda K, Iwasaki Y, Shiojima T, Kinoshita M (1996) Neuroprotective effect of various cytokines on developing spinal motoneurons following axotomy. J Neurol Sci 135:109–113
30. Ikeda K, Kinoshita M, Tagaya N, Shiojima T, Taga T, Yasukawa K, Suzuki H, Okano A (1996) Coadministration of interleukin-6 (IL-6) and soluble IL-6 receptor delays progression of wobbler mouse motor neuron disease. Brain Res 726:91–97
31. Jarskog LF, Xiao H, Wilkie MB, Lauder JM, Gilmore JH (1997) Cytokine regulation of embryonic rat dopamine and serotonin neuronal survival in vitro. Int J Dev Neurosci 15:711–716
32. Jones SA, Rose-John S (2002) The role of soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6 complex. Biochim Biophys Acta 1592:251–263
33. Kiefer R, Lindholm D, Kreutzberg GW (1993) Interleukin-6 and transforming growth factor-beta 1 mRNAs are induced in rat facial nucleus following motoneuron axotomy. Eur J Neurosci 5:775–781
34. Kuroi K, Shimozuma K, Taguchi T, Imai H, Yamashiro H, Ohsumi S, Saito S (2005) Pathophysiology of seroma in breast cancer. Breast Cancer 12:288–293
35. Kushima Y, Hama T, Hatanaka H (1992) Interleukin-6 as a neurotrophic factor for promoting the survival of cultured catecholaminergic neurons in a chemically defined medium from fetal and postnatal rat midbrains. Neurosci Res 13:267–280
36. Le Quesne PM, Fowler CJ, Harding AE (1985) A study of the effects of isaxonine on vincristine-induced peripheral neuropathy in man and regeneration following peripheral nerve crush in the rat. J Neurol Neurosur Psychiatry 48:933–935
37. Legha SS (1986) Vincristine neurotoxicity. Pathophysiology and management. Med Toxicol 1:421–427
38. Lewis ME, Neff NT, Contreras PC, Stong DB, Oppenheim RW, Grebow PE, Vaught JL (1993) Insulin-like growth factor-I: potential for treatment of motor neuronal disorders. Exp Neurol 124:73–88
39. Li M, Sendtner M, Smith A (1995) Essential function of LIF receptor in motor neurons. Nature 378:724–727
40. Marz P, Cheng JG, Gadient RA, Patterson PH, Stoyan T, Otten U, Rose-John S (1998) Sympathetic neurons can produce and respond to interleukin 6. Proc Natl Acad Sci USA 95:3251–3256
41. Matsuda S, Wen TC, Morita F, Otsuka H, Igase K, Yoshimura H, Sakanaka M (1996) Interleukin-6 prevents ischemia-induced learning disability and neuronal and synaptic loss in gerbils. Neurosci Lett 204:109–112
42. McDonald ES, Randon KR, Knight A, Windebank AJ (2005) Cisplatin preferentially binds to DNA in dorsal root ganglion neurons in vitro and in vivo: a potential mechanism for neurotoxicity. Neurobiol Dis 18:305–313
43. Mollman JE, Hogan WM, Glover DJ, McCluskey LF (1988) Unusual presentation of cis-platinum neuropathy. Neurology 38:488–490
44. Peng YP, Qiu YH, Lu JH, Wang JJ (2005) Interleukin-6 protects cultured cerebellar granule neurons against glutamate-induced neurotoxicity. Neurosci Lett 374:192–196
45. Postma TJ, Heimans JJ, Muller MJ, Ossenkoppele GJ, Vermorken JB, Aaronson NK (1998) Pitfalls in grading severity of chemotherapy-induced peripheral neuropathy. Ann Oncol 9:739–744
46. Reichert F, Levitzky R, Rotshenker S (1996) Interleukin 6 in intact and injured mouse peripheral nerves. Eur J Neurosci 8:530–535
47. Rodriguez M, Pavelko KD, McKinney CW, Leibowitz JL (1994) Recombinant human IL-6 suppresses demyelination in a viral model of multiple sclerosis. J Immunol 153:3811–3821
48. Sahenk Z, Barohn R, New P, Mendell JR (1994) Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. Arch Neurol 51:726–729
49. Saita K, Ohi T, Hanaoka Y, Furukawa S, Furukawa Y, Hayashi K, Matsukura S (1996) A catechol derivative (4-methylcatechol) accelerates the recovery from experimental acrylamide-induced neuropathy. J Pharmacol Exp Ther 276:231–237
50. Schafer KH, Mestres P, Marz P, Rose-John S (1999) The IL-6/sIL-6R fusion protein hyper-IL-6 promotes neurite outgrowth and neuron survival in cultured enteric neurons. J Interferon Cytokine Res 19:527–532
51. Taga T, Kishimoto T (1997) Gp130 and the interleukin-6 family of cytokines. Annu Rev Immunol 15:797–819
52. Toulmond S, Vige X, Fage D, Benavides J (1992) Local infusion of interleukin-6 attenuates the neurotoxic effects of NMDA on rat striatal cholinergic neurons. Neurosci Lett 144:49–52
53. Ullmann CD, Scholm J, Greiner JW (1992) Interleukin-6 increases carcinoembryonic antigen and histocompatibility

leukocyte antigen expression on the surface of human colorectal carcinoma cells. J Immunother 12:231–241
54. Wagner JA (1996) Is IL-6 both a cytokine and a neurotrophic factor? J Exp Med 183:2417–2419
55. Wang MS, Davis AA, Culver DG, Wang Q, Powers JC, Glass JD (2004) Calpain inhibition protects against Taxol-induced sensory neuropathy. Brain 127:671–679
56. Wang MS, Davis AA, Culver DG, Glass JD (2002) Wlds mice are resistant to paclitaxel (Taxol) neuropathy. Ann Neurol 52:442–447
57. Yamada M, Hatanaka H (1994) Interleukin-6 protects cultured rat hippocampal neurons against glutamate-induced cell death. Brain Res 643:173–180
58. Yoshida K, Taga T, Saito M, Suematsu S, Kumanogoh A, Tanaka T, Fujiwara H, Hirata M, Yamagami T, Nakahata T, Hirabayashi T, Yoneda Y, Tanaka K, Wang WZ, Mori C, Shiota K, Yoshida N, Kishimoto T (1996) Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. Proc Natl Acad Sci USA 93:407–411
59. Ziegler D, Reljanovic M, Mehnert H, Gries FA (1999). Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials. Exp Clin Endocrinol Diabetes 107:421–430