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Effect of the chronic combined administration of cisplatin and paclitaxel in a rat model of peripheral neurotoxicity

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ABSTRACT

We have characterised for the first time the general and neurological side effects experienced when using a series of chronic non-lethal cisplatin + paclitaxel schedules in Wistar rats, selected according to our previous experience and the animals' maximum tolerated dose.

At the pathological level, the use of combination schedules was definitely more toxic at the kidney and sternal bone marrow level than the single-agent schedules.

At the neurophysiological examination based on the assessment of the nerve conduction velocity measurement in the tail nerve, we identified only one combination schedule that was more neurotoxic than the similar schedules based on single-agent administration. This observation was confirmed by the neuropathological examination performed on the sciatic nerve, dorsal root ganglia, ventral and dorsal roots.

Our study supports the hypothesis that the general and, to a lesser extent, neurological effects of a combination of cisplatin and paclitaxel are different from those of the administration of both drugs as single agents. We believe that these models may be useful for testing neuroprotective strategies.

1. Introduction

Chemotherapy-induced peripheral neuropathy is amongst the most severe and limiting side effects of several widely used anticancer compounds.[1]

Several *in vivo* single-agent models of chemotherapy-induced peripheral neurotoxicity have been developed in recent years and they have focused on the most widely used neurotoxic compounds.[2,3] However, combination schedules including more than one neurotoxic agent are commonly used. This is the case with the platinum-taxane combination for the treatment of several solid malignancies, but despite its widespread use no detailed chronic platinum-taxane combination models have so far been reported.

In this study, we have established and characterised for the first time the general and neurological side effects of a series of chronic cisplatin + paclitaxel schedules in Wistar rats at the neurophysiological, pathological and haematological levels.

2. Materials and methods

Young adult female Wistar rats (175–200 g, Harlan Italy, Correzzana) were used for the study. The care and husbandry of the animals were in conformity with the institutional guidelines in compliance with national (D.L. n. 116, *Gazzetta Ufficiale della Repubblica Italiana*, suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1,

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Dec.12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

2.1. Drugs and schedules of administration

The study was divided into two parts (Table 1). Doses of cisplatin and paclitaxel were selected according to our previous experience[2,3] and to the animals' maximum tolerated dose. Cisplatin (Platamine, Pfizer, Nerviano, Italy) was dissolved in sterile saline, while paclitaxel (LC Laboratories, Woburn, MA) was dissolved in absolute ethanol/Tween 80/saline (5/5/90%).

Cisplatin was administered intraperitoneally (ip) and paclitaxel intravenously (iv) via the tail vein.

In order to mimic as carefully as possible the clinical use of the two drugs, in all combination schedules one drug was administered 1 h before the second compound.

2.1.1. Experiment 1

In order to define the effect of the weekly schedule of the selected doses of cisplatin, two groups of rats were treated with cisplatin 3 or 4 mg/kg weekly × 4 weeks (Groups 1 and 2, 17.7 and 23.6 mg/m^2 weekly respectively); paclitaxel 5 or 10 mg/kg weekly × 4 weeks was administered to two other groups of rats (Groups 3 and 4, 29.5 and 59.0 mg/m^2 weekly respectively) and used as a reference for the combination schedules. Low-intensity combination schedules were also evaluated using paclitaxel 5 mg/kg + cisplatin 3 mg/kg, paclitaxel 5 mg/kg + cisplatin 4 mg/kg or paclitaxel 10 mg/kg + cisplatin 3 mg/kg (Groups 5, 6 and 7 respectively).

2.1.2. Experiment 2

Based on the results of the dose-finding study, the combination of paclitaxel 10 mg/kg and cisplatin 4 mg/kg was also investigated and both sequences were evaluated (Groups 8 and 9). Rats treated with cisplatin 3 or 4 mg/kg (Groups 1 and 2), with paclitaxel 10 mg/kg + cisplatin 3 mg/kg (Group 7) or with paclitaxel 10 mg/kg (Group 4) were used as a reference in Experiment 2, as well as untreated control rats. Some of the rats in this experiment belonging to Groups 2, 4, 8, 9 and controls underwent a 6-week follow-up period of observation after drug treatment withdrawal.

2.2. Clinical signs and body weight (Experiments 1 and 2)

The general clinical condition of the animals was assessed daily. Body weight was recorded before each drug administration for drug dose adjustment, on the days of scheduled sacrifices and at the 4th week in the follow-up period.

2.3. Neurophysiological assessment (Experiments 1 and 2)

At baseline, after treatment and, only in Experiment 2, after 4 and 6 weeks in the follow-up period, the nerve conduction velocity (NCV) was determined in the tail nerve of each animal as previously described.[2,4] In brief, the NCV was assessed by placing recording ring electrodes distally in the tail, while the stimulating ring electrodes were placed 5 cm and 10 cm proximally with respect to the recording point. The latencies of the potentials recorded at the two sites after nerve stimulation were determined (peak-to-peak) and the NCV was calculated accordingly. All the neurophysiological determinations were performed under standard conditions in a temperature-controlled room.

2.4. Haematological and haemato-chemical determinations (Experiment 1)

Group 5, 6 and 7 rats and controls were used for haematological and haemato-chemical determinations in order to estimate the general toxicity of low-intensity combination schedules in Experiment 1 and to better plan Experiment 2.

Blood samples were collected from the tail vein into K2EDTA test tubes for haematological determinations (Advia 120 - Bayer) and into no additive test tubes to obtain serum for AST, ALT, BUN and creatinine determination (Cobas Mira Classic - Roche).

Table 1 – Summary of the schedules used in the study.

Experiment 1 Groups	Schedules, weekly administration × 4 (N. of animals/group)	Follow-up period
Group 1 (c3)	CDDP ip 3 mg/kg (8)	–
Group 2 (c4)	CDDP ip 4 mg/kg (8)	–
Group 3 (p5)	PACLI iv 5 mg/kg (8)	–
Group 4 (p10)	PACLI iv10 mg/kg (8)	–
Group 5 (p5 + c3)	PACLI iv 5 mg/kg + CDDP ip 3 mg/kg (8)	–
Group 6 (p5 + c4)	PACLI iv 5 mg/kg + CDDP ip 4 mg/kg (8)	–
Group 7 (p10 + c3)	PACLI iv 10 mg/kg + CDDP ip3 mg/kg (8)	–
Controls	Untreated animals (8)	–
Experiment 2 Groups	**Schedules, weekly administration × 4 (N. of animals/group)**	**Follow-up period (N. of animals/group)**
Group 1 (c3)	CDDP ip 3 mg/kg (6)	–
Group 2 (c4)	CDDP ip 4 mg/kg (12)	6 weeks (6)
Group 4 (p10)	PACLI iv 10 mg/kg (12)	6 weeks (6)
Group 7 (p10 + c3)	PACLI 10 iv mg/kg + CDDP ip 3 mg/kg (6)	–
Group 8 (p10 + c4)	PACLI 10 iv mg/kg + CDDP ip 4 mg/kg (12)	6 weeks (6)
Group 9 (c4 + p10)	CDDP ip 4 mg/kg + PACLI iv 10 mg/kg (12)	6 weeks (6)
Controls	Untreated animals (12)	6 weeks (6)

2.5. Post-mortem histopathological examination (Experiment 2)

At the end of the treatment period (Groups 1, 2, 4, 7, 8, 9 and controls) or after the 6-week follow up period (only from Groups 2, 4, 8, 9 and controls), rats were perfused under general anaesthesia with paraformaldehyde 0.12 M in phosphate buffer solution (pH = 7.4).

The left sciatic nerves, the L4-L5 ventral and dorsal roots and the L4-L5 dorsal root ganglia (DRG) were post-fixed in OsO_4, epoxy resin embedded and used for light and electron microscope observations. Toluidine blue stained semithin (1 μm-thick) sections were prepared from at least two tissue blocks for each animal and examined with a Nikon Coolscope light microscope. Ultrathin sections counterstained with uranyl acetate and lead citrate were examined with a Philips CM 10 transmission electron microscope. Kidney and decalcified-sternum specimens were post-fixed in buffered 10% formalin, paraffin embedded, stained with haematoxilin-eosin and examined with a Nikon Coolscope light microscope.

2.6. Morphometric analysis on DRG (Experiment 2)

DRG neurons obtained after the last administration from rats belonging to Groups 1, 2, 4, 7, 8, 9 and controls underwent morphometric examination. The somatic, nuclear and nucleolar size of DRG neurons were measured in randomly selected sections according to previously reported methods on at least 300 DRG neurons/rat.[3]

2.7. Statistical evaluation

Body weight, NCV and morphometric data were statistically evaluated using the analysis of variance (one-way ANOVA) and the Tukey–Kramer post-test (significant if $p < 0.05$). Although Figs. 1 and 3 report the normalised values versus the control untreated rats of each experiment, the results of the statistical analysis refer to the evaluation performed on the original raw values.

3. Results

3.1. General toxicity

3.1.1. Clinical signs and mortality

The administration of cisplatin or paclitaxel alone or in combination was generally well tolerated by the rats and only a small number of animals treated with a high dose of cisplatin presented kyphoses, hypokinesia and piloerection. No mortality was observed in any of the treated groups.

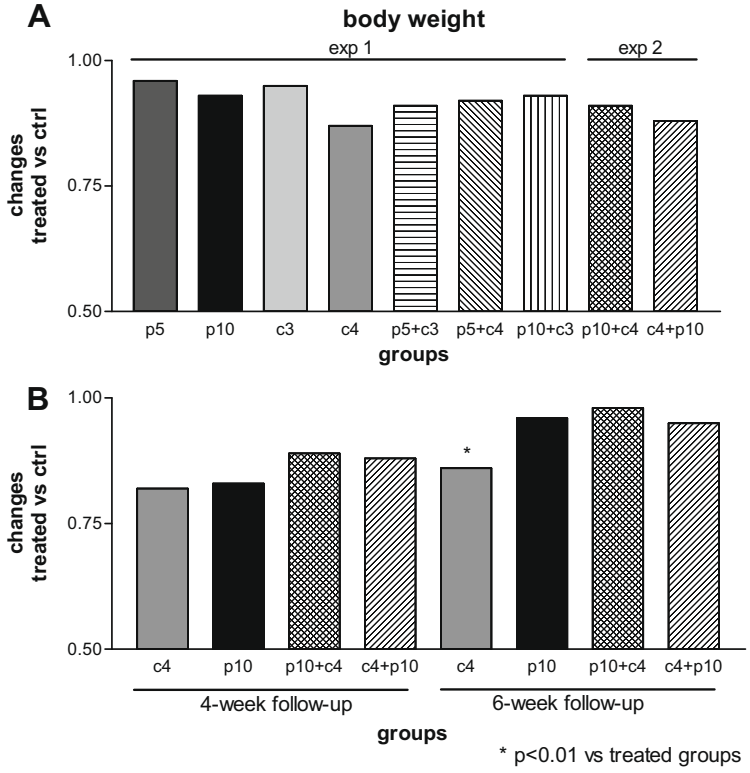

Fig. 1 – Drawing of the percentage changes in treated groups versus untreated controls (Y-axis value for control group = 1) in body weight at the end of the treatment phase (A, Experiments 1 and 2 as indicated) and after the follow-up (B, Experiment 2). All the treated groups had a significant decrease in body weight at the end of the treatment phase and at the 4-week follow-up evaluation. Recovery occurred at the end of the follow-up period, the only exception being the group treated with the high dose single-agent cisplatin schedule (c4, *). Bars refer to the changes versus the untreated controls of the mean value of the respective experiment. Statistical significance is calculated on the original raw values. Groups: c = cisplatin, p = paclitaxel, Doses: 3 = 3 mg/kg, 4 = 4 mg/kg, 5 = 5 mg/kg, 10 = 10 mg/kg.

3.1.2. *Weight changes*

Weight changes are reported in Fig. 1. All the treatments induced a significant body weight reduction versus control rats in both experiments ($p < 0.01$ in all cases). At the inter-group comparison among combination schedules, no significant differences were observed among groups and versus single-agent treatments. In the follow-up period, after an initial phase where the difference versus untreated controls further increased ($p < 0.01$ in all cases), body weight recovery occurred in all the treated groups available for comparison with the only exception being the Group 2 rats treated with cisplatin 4 mg/kg which, at the 6-week follow up evaluation, still had a significant weight gain impairment versus untreated controls ($p < 0.01$, Fig. 1).

3.1.3. *Haematological and serum chemistry determinations*

Treated groups (5, 6 and 7) showed moderate to marked lymphocytic leucopoenia, this being more severe in the group treated with the highest dose of paclitaxel, with a normal red blood cell count.

No significant changes were observed in the treated groups versus controls in kidney and liver function (data not shown).

3.1.4. *Target organ histopathology*

In Experiment 2 a morphological evaluation of target organs was microscopically carried out on kidney and sternal bone marrow (Table 2 and Fig. 2).

As expected, cisplatin administered as a single agent at both dose-levels in Groups 1 and 2 (i.e. 3 and 4 mg/kg) induced

Table 2 – Histopathology results on kidney and sternal bone marrow – Experiment 2.

Group	After treatment (6 rats/group)		After follow-up (6 rats/group)	
	Kidney (tubulopathy)	Bone marrow (hypoplasia)	Kidney (tubulopathy)	Bone marrow (hypoplasia)
Group 1 (c3)	++	+	ND	ND
Group 2 (c4)	++/+++	+/++	++++/+++++	+/++
Group 4 (p10)	nrf	nrf	nrf	nrf
Group 7 (p10 + c3)	++/+++	++	ND	ND
Group 8 (p10 + c4)	+++/++++	++/+++	+++/++++	++
Group 9 (c4 + p10)	+++/++++	+++/++++	++++/+++++	++

Score: + = minimal; ++ = slight; +++ = moderate; ++++ = marked; +++++ = severe; nrf = no remarkable findings; ND = not determined (arbitrary score assessed by a blinded, certified animal pathologist).

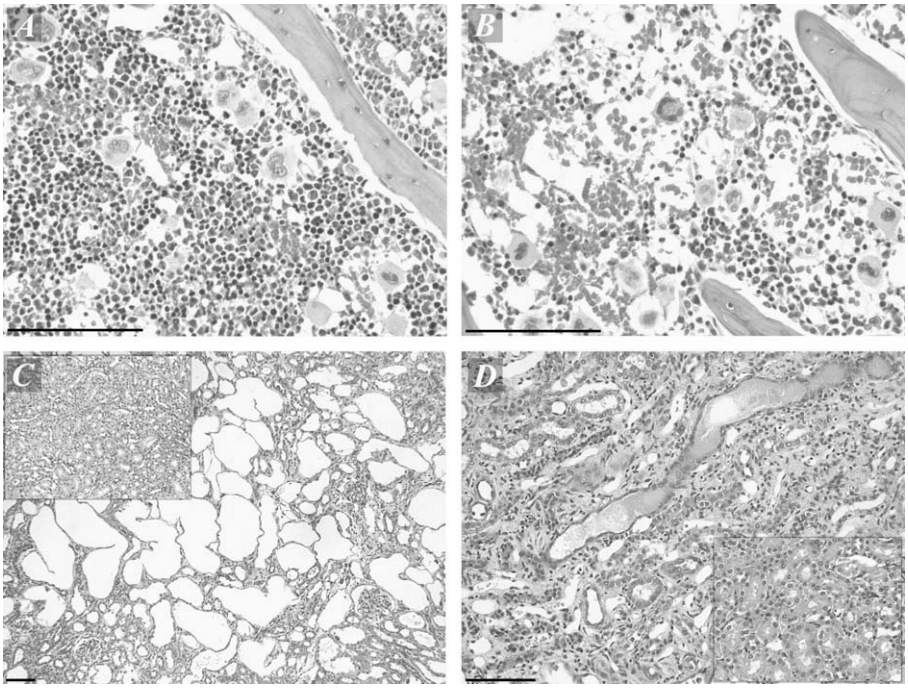

Fig. 2 – Histopathology – toxic changes. (A) sternal bone marrow: control – normal cellularity. (B) sternal bone marrow (paclitaxel 10 mg/kg + cisplatin 4 mg/kg) showing severe hypoplasia (decreased cellularity) with dilation of capillary vessels and focal haemorrhages. (C) kidney – outer strip of outer medulla (paclitaxel 10 mg/kg + cisplatin 4 mg/kg) with severe tubulopathy (cystic tubular dilation); insert shows a section obtained from a control rat. (D) kidney – outer strip of outer medulla (paclitaxel 10 mg/kg + cisplatin 4 mg/kg) showing marked tubulopathy (severe diffuse tubular degeneration/regeneration, irregular tubular lumens lined by dysplastic epithelium, interstitial fibrosis with inflammatory infiltration); insert shows a section obtained from a control rat (bars = 100μm).

dose-dependent and progressive chronic tubulopathy in the kidney and myelosuppression in the sternal bone marrow which were still present after the recovery period. Treatment related nephrotoxic changes were represented by degenerative/regenerative chronic tubulopathy characterised by tubular necrosis with disepithelisation, tubular dilation, tubular hyperplasia/basophilia, interstitial inflammatory infiltration and interstitial fibrosis. These changes were more evident in the proximal tubular component of the outer medulla and inner cortex. Treatment related myelotoxic changes in sternal bone marrow were represented by hypoplasia (i.e. decreased cellularity) with dilation of capillary vessels and focal haemorrhages.

Paclitaxel as a single agent (Group 4, 10 mg/kg) did not induce any evident lesions in kidney and bone marrow.

Cisplatin (3 and 4 mg/kg) and paclitaxel (10 mg/kg) administered in combination in Groups 7, 8 and 9 induced an evident cumulative toxicity with a worsening of nephropathy and myelosuppression versus single-agent treatments (Table 2).

3.2. *Neurotoxicity*

3.2.1. *Neurophysiological evaluation*

The results obtained in the study are reported in Fig. 3. After treatment a significant difference ($p < 0.01$, Fig. 3A) was observed in all groups versus controls in both experiments. The inter-group comparison between single-agent and combination schedules evidenced that the combination paclitaxel 5 mg/kg + cisplatin 4 mg/kg (Group 6) was significantly more neurotoxic than the administration of paclitaxel 5 mg/kg alone (Group 3, $p < 0.01$). In Experiment 2, no sequence-dependent difference was observed immediately after treatment between the NCV results obtained in the groups treated with paclitaxel 10 mg/kg combined with cisplatin 4 mg/kg (Groups 8 and 9). Moreover, the severity of the neurotoxicity was not significantly different from that observed in the rats treated with each single-agent at the same high dose (Groups 2 and 4). However, at the evaluation performed after the 4-week follow-up period, recovery was incomplete in Group 8 ($p < 0.05$ versus controls, Fig. 3B) while no significant difference was observed in the remaining groups, and only after an additional 2-week period of observation was complete recovery demonstrated by the statistical evaluation.

3.2.2. *Neuropathological examination*

The pathological observations were performed in Experiment 2 on DRG, ventral and dorsal roots and sciatic nerve specimens obtained after treatment and after the follow-up period from rats belonging to Groups 1, 2, 4, 7, 8 and 9 and from controls.

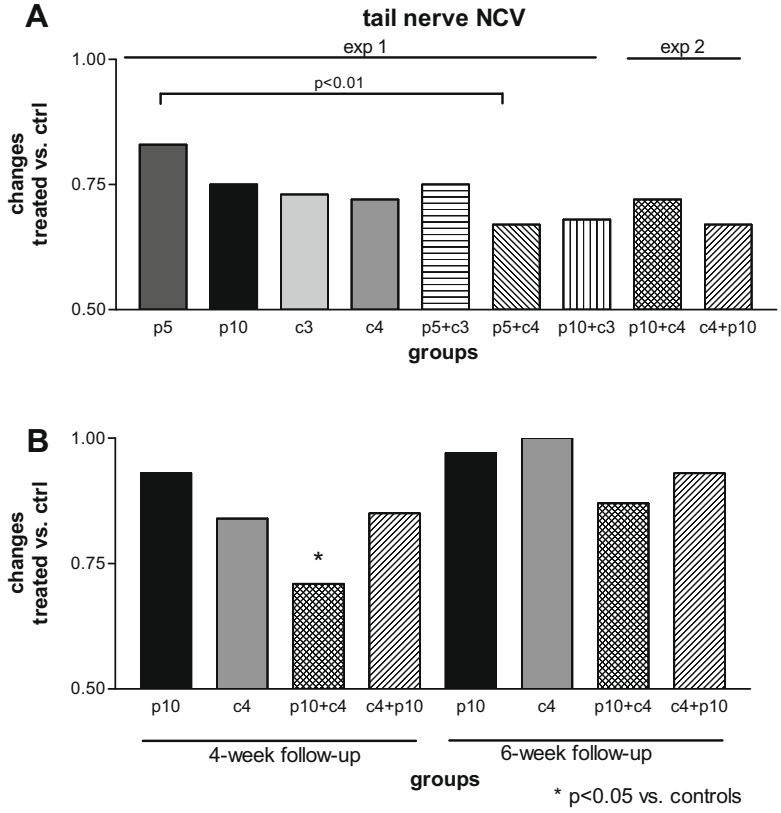

Fig. 3 – Drawing of the percentage changes in treated groups versus untreated controls (Y-axis value for control group = 1) in nerve conduction velocity (NCV) at the end of the treatment phase (A, Experiments 1 and 2 as indicated) and after the follow-up (B, Experiment 2). All the treated groups had a significant decrease in NCV at the end of the treatment phase while recovery occurred after the 6-week follow-up period. Bars refer to the changes versus the untreated controls of the mean value of the respective experiment. Statistical significance is calculated on the original raw values. Groups: c = cisplatin, p = paclitaxel, Doses: 3 = 3 mg/kg, 4 = 4 mg/kg, 5 = 5 mg/kg, 10 = 10 mg/kg.

At the light microscope examination performed after treatment, DRG neurons looked smaller in size in cisplatin-treated groups (Groups 1 and 2) than in controls, and multinucleolated neurons with eccentric nucleoli were present, but no other evidence of cell damage was observed. The satellite cells had a normal appearance. The electron microscopic examination performed on DRG specimens obtained from cisplatin-treated rats evidenced nucleolar segregation in several neuron nucleoli, while cytoplasmatic changes were absent. At the light microscope no differences were evident between the cisplatin alone and any of the paclitaxel + cisplatin-treated rats (Groups 7, 8 and 9). Also, the ultrastructural examination did not evidence any relevant morphological differences between the rats treated with cisplatin alone or in combination with paclitaxel. No obvious pathological changes were observed in the neurons or satellite cells in DRG obtained from rats treated with paclitaxel alone (Group 4). At the follow-up examination performed on DRG specimens belonging to rats of Groups 2, 4, 8 and 9, no pathological changes were still evident.

After treatment, the light and electron microscopic examinations revealed an axonopathy in the sciatic nerve of rats belonging to all the treated groups. The extent of axonopathy was slightly more severe in the groups treated with the high intensity combination schedules (Groups 8 and 9, Fig. 4). Recovery was observed after the 6-week follow-up period in Groups 2, 4, 8 and 9. The administration of paclitaxel induced the occurrence of polymerised tubules in the axons of large and small fibres, and no difference was observed in the groups treated with paclitaxel alone or in combination with cisplatin.

In the ventral roots, mild axonal changes were observed in all the paclitaxel-treated groups and these changes were not more frequent in the groups co-treated with cisplatin (Fig. 5). In the dorsal roots, only very rarely were degenerating fibres present in any of the treated groups while the overall aspect of the fibre population was normal (Fig. 5).

3.2.3. *Morphometric analysis on DRG neurons*

The morphometric analysis carried out on the DRG obtained from rats belonging to Groups 1, 2, 4, 7, 8, 9 and controls did not demonstrate any change in somatic, nuclear and nucleolar size in the Group 4 rats treated with paclitaxel 10 mg/kg alone versus controls. On the contrary, the administration of cisplatin alone (Groups 1 and 2), or of the paclitaxel + cisplatin combinations, consistently induced a significant reduction in somatic, nuclear and nucleolar size ($p < 0.01$ in all cases) the only exception being the Group 7 rats treated with paclitaxel 10 mg/kg + cisplatin 3 mg/kg which displayed the expected reduction in somatic ($p < 0.01$), but not in nuclear and nucleolar, size (Fig. 6).

4. Discussion

In order to investigate the mechanisms of the toxicity of platinum drugs and taxanes and to test neuroprotective strategies, several single-agent well-characterised animal models have been established.[4–7] The effect of the combined administration of the two first-in-class compounds (i.e. cisplatin and paclitaxel) has been explored *in vivo* in only one animal model in which the administration of paclitaxel before cisplatin was much less neurotoxic than the opposite sequence

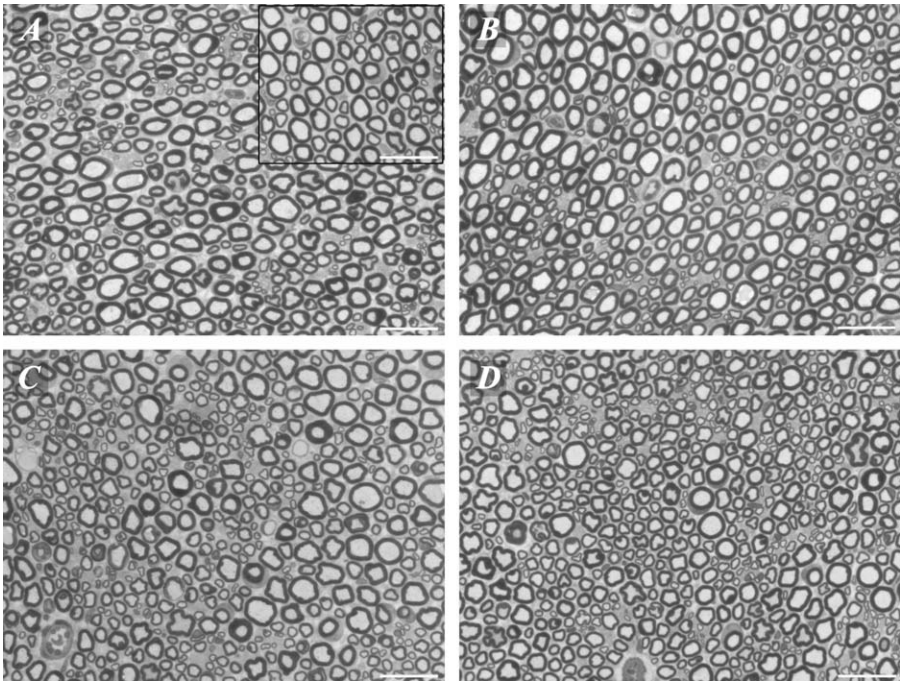

Fig. 4 – Sciatic nerve light photomicrographs from specimens obtained after treatment, showing in all the cases mild axonopathy (A = cisplatin 4 mg/kg, B = paclitaxel 10 mg/kg, C = paclitaxel 10 mg/kg + cisplatin 4 mg/kg, D = cisplatin 4 mg/kg + paclitaxel 10 mg/kg; insert in A = control). Bar = 50 μm.

of treatment, thus suggesting a neuroprotective effect of sequencing.[8] However, that experimental design did not match the time course of drug administration in humans since the interval between the administration of the two drugs was much longer (i.e. 24 h) than in clinical practice. Moreover, no information regarding the general toxicity of the treatment schedule and the follow-up course of the neuropathy was reported.

In our study, cisplatin administration was associated with a rather severe effect on body weight. Moreover, body weight recovery was not achieved during the follow-up in the group treated with the high dose cisplatin schedule, although the animals survived until the end of the 6-week follow-up period. An intriguing observation is that the co-administration of paclitaxel completely prevented this detrimental effect of cisplatin on body weight recovery.

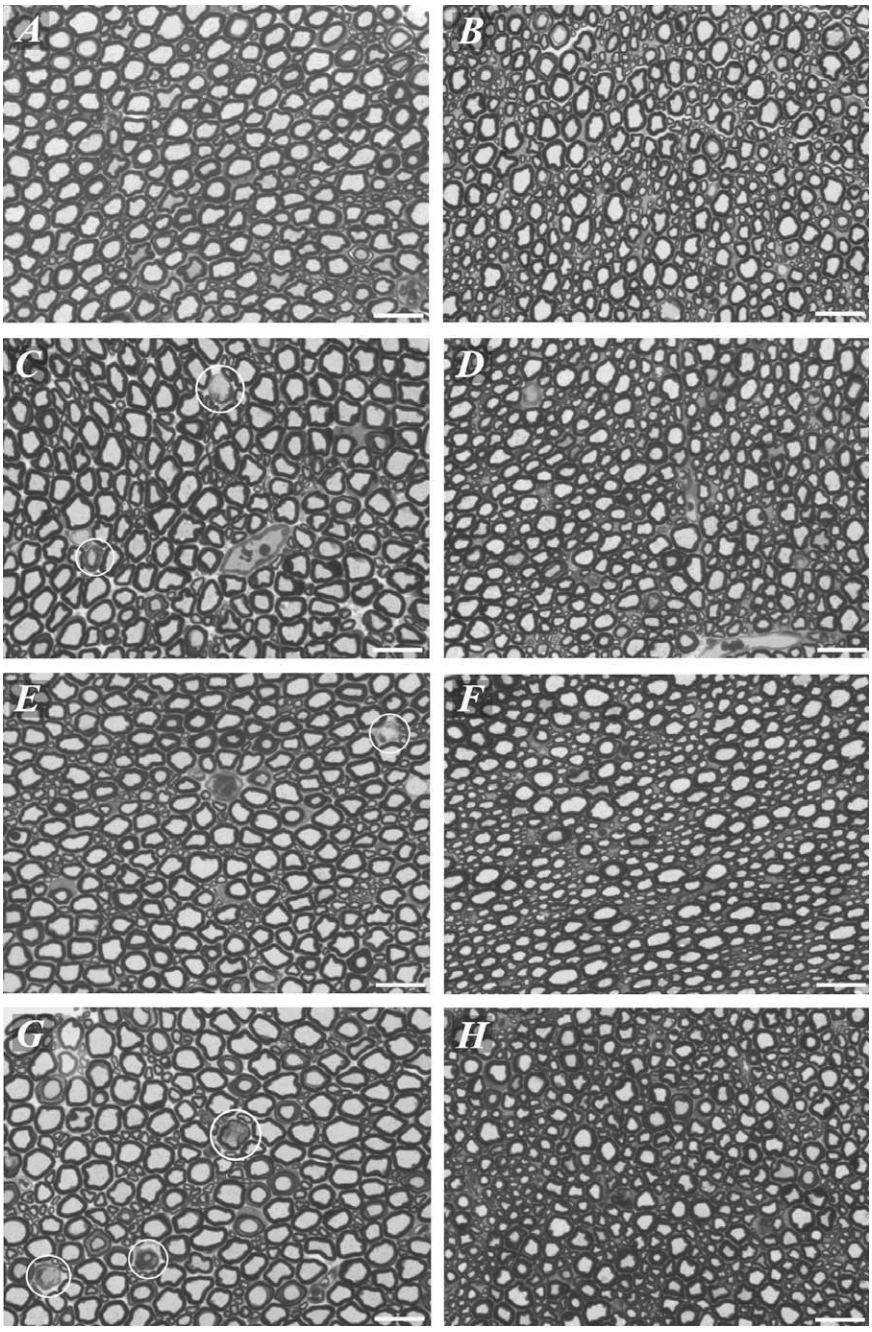

Fig. 5 – Ventral root light photomicrographs from specimens obtained after treatment, showing mild axonopathy (circle) in the rats treated with paclitaxel (A = cisplatin 4 mg/kg, C = paclitaxel 10 mg/kg, E = paclitaxel 10 mg/kg + cisplatin 4 mg/kg, G = cisplatin 4 mg/kg + paclitaxel 10 mg/kg). In dorsal root specimens obtained after treatment normal aspect of myelinated fibres was generally observed (B = cisplatin 4 mg/kg, D = paclitaxel 10 mg/kg, F = paclitaxel 10 mg/kg + cisplatin 4 mg/kg, H = cisplatin 4 mg/kg + paclitaxel 10 mg/kg). Bar = 50 μm.

Target organ histopathology demonstrated the well known nephrotoxic and myelotoxic effects induced in the rat by cisplatin when administered as a single agent[9,10] while it confirmed the lack of paclitaxel-induced toxic effects on the kidney.[11] The co-administration of paclitaxel + cisplatin enhanced the toxicity in the kidney and sternal bone marrow demonstrating a significant synergism in the toxic activity of combination. Interestingly, toxic synergism was less evident when paclitaxel was administered before cisplatin. All these pathological changes were still clearly evident (particularly in the kidney) after the 6-week follow up period, i.e. when recovery from the peripheral neurotoxicity was evident, thus ruling out the possibility that the neurotoxic effects observed on the DRG and peripheral nerves are simply due to the general toxicity of the compounds or of their combination. In the group treated with high dose cisplatin, kidney pathological changes markedly increased after the 6-week follow-up period; this further deterioration was not evident in the groups co-treated with paclitaxel, an observation that might be at the basis of the body weight course previously discussed.

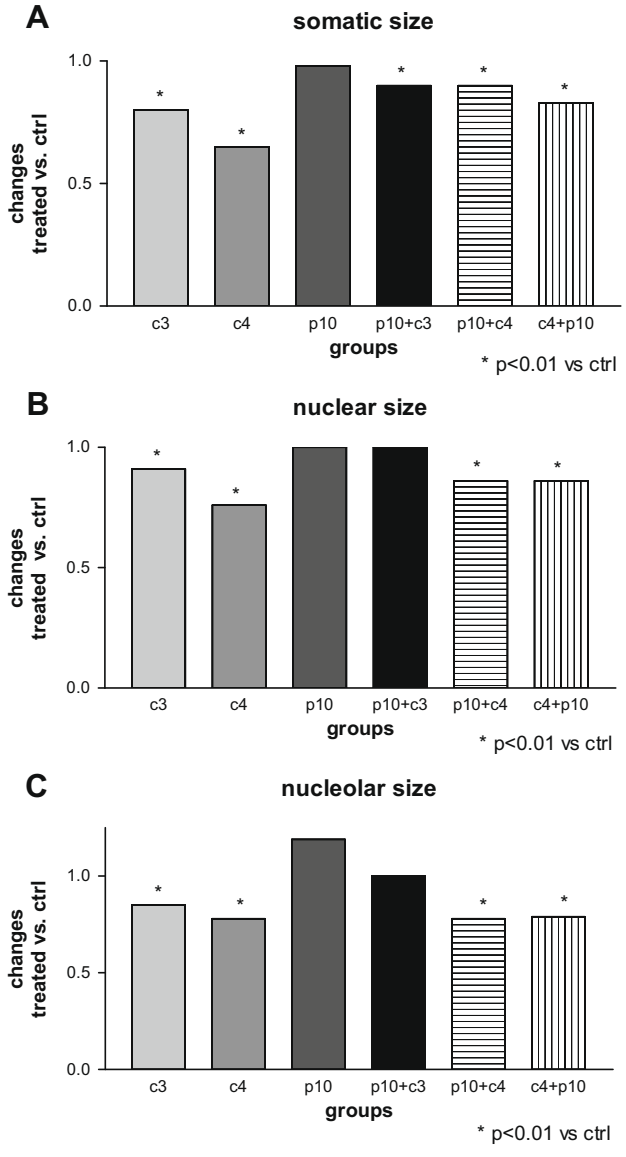

Fig. 6 – Drawing of the percentage changes in treated groups versus untreated controls (Y-axis value for control group = 1) in somatic (A), nuclear (B) and nucleolar (C) size at the end of the treatment phase in Experiment 2. Statistical significance is calculated on the original raw values. Groups: c = cisplatin, p = paclitaxel, Doses: 3 = 3 mg/kg, 4 = 4 mg/kg, 5 = 5 mg/kg, 10 = 10 mg/kg.

A significant reduction in NCV was observed in all the treated groups which is in agreement with our previous data on paclitaxel and with those obtained in the cisplatin models based on the administration of the drug twice weekly (within the same total dose range used in this study).[2,4] When single-agent versus combination schedules were compared after treatment, an increased severity of the neurotoxic effect was evidenced only when the low dose of paclitaxel (i.e. 5 mg/kg) combined with the high dose of cisplatin (i.e. 4 mg/kg) was compared with single-agent administration. No difference was observed when the two different high dose cisplatin-paclitaxel sequences were compared.

The neuropathological examination evidenced mild axonal changes in the sciatic nerve and in the ventral roots. This latter observation was more evident in paclitaxel-treated rats (alone or in combination), thus confirming previous observations obtained using high dose paclitaxel administration in rats.[12] No clear-cut pathological changes were present in the dorsal roots of rats belonging to any treated group. In the DRG neurons, only the typical changes already reported in cisplatin-treated rats were present and they were also observed in the rats that underwent combination treatment. At the morphometric level, when the low and less neurotoxic dose of cisplatin was combined with paclitaxel, an interesting effect occurred on nuclear and nucleolar size. In fact, no nuclear or nucleolar size reduction was observed in comparison with control rats. This effect was no longer evident when the high, more neurotoxic dose of cisplatin was administered. Our results, suggesting an interference by paclitaxel on cisplatin-induced nuclear and nucleolar effects, are in agreement with previous data limited to the nucleolus.[8] An effect of paclitaxel on nucleolar size was also suggested by the experiments reported by Jamieson and colleagues after a single administration of paclitaxel + oxaliplatin[13] or after prolonged paclitaxel administration (in this latter case only on a subpopulation of large DRG neurons).[14]

The 'classical' targets of the neurotoxicity of cisplatin and paclitaxel have been well established (i.e. DRG neurons and axonal tubulin, respectively). However, it has been reported that tubulin can also be targeted by cisplatin[15], as well as the fact that DRG changes can be induced by paclitaxel[14,16], although they are not evident at the routine histopathological examination at light and ultrastructural examination. Another possible, although less well-documented, common site of action of the two compounds is represented by mitochondria. Evidence that mitochondrial dysfunction can be induced by cisplatin (probably through binding to mitochondrial DNA) has recently been reported[17,18], although a direct confirmation in chronic *in vivo* peripheral neurotoxicity models has not yet been achieved. Similarly, paclitaxel-induced mitochondrial damage has been demonstrated in an animal model of painful neuropathy.[5,7] Interestingly, neuroprotection from

cisplatin and paclitaxel neurotoxicity was achieved in different models[4,7] by acetyl-L-carnitine administration, and one of the postulated mechanisms of action of the protective drug is an effect on mitochondrial activity. Moreover, several antioxidant treatments can reduce cisplatin and paclitaxel neurotoxicity, and it can be hypothesized that they prevent oxidative stress secondary to reduced mitochondrial activity and energy failure.[19–21] It is, however, possible that the two antineoplastic compounds share some other still unidentified common site/mechanism of action. For instance, several alterations in gene expression have been reported after cisplatin[22–25] or paclitaxel[26,27] administration, although no common gene expression change has so far been identified.

All these experimental observations imply that the use of the combination of the two compounds might be potentially more nephrotoxic, myelotoxic and neurotoxic than the administration of each drug alone. Regarding neurotoxicity, this theoretical assumption has never been clearly established. For instance, we demonstrated an unexpectedly severe neurotoxicity of paclitaxel administration in patients with a relapse of ovarian cancer who had been pre-treated with cisplatin.[28] However, when we compared the effect of cisplatin + cyclophosphamide versus cisplatin + paclitaxel used as first-line schedules in a similar series of ovarian cancer patients, the overall neurotoxicity was not significantly different between the two groups.[29] In our experiments, the increase in peripheral neurotoxicity was observed in only one of the schedules examined, while the high-dose combinations were not significantly more neurotoxic than each high-dose, non-lethal schedule of both single agents. However, the recovery in the follow-up period was significantly delayed in the group treated with paclitaxel 10 mg/kg + cisplatin 4 mg/kg. In terms of general toxicity, the combination schedules were clearly more neurotoxic than each single-agent administration although, to clearly assess this effect, organ histopathology was required, since blood testing was not informative enough.

Finally, in our study we did not evidence any effect of drug sequencing on the peripheral neurotoxicity of paclitaxel and cisplatin. This possibility was raised by the pre-clinical *in vivo* results reported by McKeage and colleagues[8] and it was hypothesised that it was due to the opposite effect of the two drugs on tubulin dynamic equilibrium, although no evidence for this hypothesis was provided. When we investigated this issue in an *in vitro* model, no change in paclitaxel-induced tubulin polymeration was induced by the combination with cisplatin.[30]

In conclusion, we have characterised the general and neurological toxicity of a high-dose cisplatin weekly schedule, and we have described in detail the effect and time course of several low- and high-dose paclitaxel + cisplatin schedules. These combination models, which allow the survival of the rats with acceptable general toxicity, may be useful for testing neuroprotective strategies and for investigating the mechanisms of action of both antineoplastic drugs.

Conflict of interest statement

None declared.

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