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Cisplatin-induced peripheral neuropathy: Neuroprotection by erythropoietin without affecting tumour growth

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ABSTRACT

This study examined the dose-dependent efficacy of erythropoietin (EPO) for preventing and/or treating cisplatin (CDDP) induced peripheral neurotoxicity (CINP), and its influence on tumour treatment and growth. Rats received eight intraperitoneal (ip) injections of 2 mg/kg CDDP twice weekly. EPO co-administered (50 or 10 μg/kg ip, three times/week) had a dose-dependent effect, partially preventing CINP, but 0.5 μg/kg ip was not effective. The neuroprotective effect lasted at least 5 weeks after the last dose of EPO and CDDP. In addition, EPO (50 μg/kg ip three times/week) after the last injection of CDDP still induced a significant recovery of CINP. In a separate experiment in rats bearing mammary carcinoma EPO treatment (50 μg/kg ip) given concurrently with CDDP (1.0 and 1.5 mg/kg twice a week for four weeks) was neuroprotective without influencing the effectiveness of the treatment or tumour growth. EPO thus appears to be an effective neuroprotectant that does not interfere with tumour treatment.

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1. Introduction

Erythropoietin (EPO) is a cytokine originally used for its effect on erythropoiesis, since it supports the survival, proliferation and differentiation of erythroid progenitor cells. However, in the past few years it has become clear that EPO is a multifunctional trophic factor[1–6] with potent neurotrophic activity on a variety of neural cells in the central and peripheral nervous system.[7–11] EPO acts by binding with its receptors (EPOR), cytokines belonging to the receptor type I superfamily.[3,12,13] EPOR are expressed in nerve axons, in Schwann cells and in dorsal root ganglia. They are over-expressed after nerve injury, which is the basis for therapeutic use of exogenous EPO.[3,5,14] *In vivo* experimental models allowed to demonstrate that EPO can both prevent and treat diabetic peripheral neuropathy.[15] Several chemotherapeutic drugs, including cisplatin (CDDP),

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induce peripheral neurotoxicity (CINP), with a dose-limiting effect and possible impairment of the quality of life of cancer patients.[16] We have developed an experimental model of peripheral neurotoxicity induced by CDDP that closely resembles CINP.[17–22] EPO can also partly prevent the onset of CDDP neurotoxicity when the two drugs are co-administered.[23,24]

With the aim of clarifying the potential neuroprotectant role for EPO, we investigated whether a dose-response effect could be identified and EPO could 'cure' CINP when administered after CDDP, and not only 'preventing' it when co-administered. Finally, we checked whether EPO interfered with CDDP's antitumour activity.

2. Materials and methods

2.1. *Animal husbandry*

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines in compliance with national (Law by Decree No. 116, February 18, 1992, *Gazzetta Ufficiale della Repubblica Italiana*, Suppl. 40) and international laws and policies (European Economic Community Council Directive 86_609, December 12, 1987, in *Official Journal of Law*, p. 358; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The protocols for the proposed investigation were reviewed and approved by the Animal Care and Use Committees of the Istituto di Ricerche Farmacologiche 'Mario Negri' (Milan) and the Faculty of Medicine, University of Milano 'Bicocca'.

Animals were housed in a limited access animal facility. Animal room temperature and relative humidity were set at 22 ± 2 °C and 55 ± 10%. Artificial lighting provided a 12 hours light/12 hours dark cycle (7 a.m.–7 p.m.).

2.2. *Experiment 1: treatments*

Sixty-two female Wistar rats (200–220 g at the beginning of the experiment, Harlan Italia, Correzzana, Italy) were used. They were divided into six groups (number of animals/group): untreated controls (CTRL; 8), high-dose EPO controls (CTRL + EPO 50, 50 μg/kg intraperitoneal - ip - three times/week; 8), CDDP (2 mg/kg twice weekly × 4 wks; 22), CDDP + high-dose EPO (CDDP + EPO 50, 50 μg/kg ip three times/week; 8), CDDP + intermediate-dose EPO (CDDP + EPO 10, 10 μg/kg ip three times/week; 8), CDDP + low-dose EPO (CDDP + EPO 0.5, 0.5 μg/kg ip three times/week; 8).

CDDP (*Platamine*, Pharmacia Italia, Milan, Italy) was dissolved in sterile saline and rats were injected 2 mg/kg ip twice/weekly eight times using a volume of 4 mL/kg.[24–26] EPO (Epoietin alfa, recombinant human EPO, kindly donated by Ortho Biotech, a Janssen-Cilag division, Milan, Italy) was delivered in the appropriate dose using pre-filled syringes. The CTRL rats received sham ip injections of the CDDP solvent.

At the end of the treatments, eight rats from the CDDP group were given the high dose of EPO 50 μg/kg ip three times/week, for 5 weeks, indicated as therapeutic schedule (CDDP + EPO 50 ther.). The flow-chart of the study is reported in Fig. 1.

2.3. *Experiment 2: treatments*

Female Fisher 344 rats (125–150 g at the beginning of the experiment, Charles River, Calco, Italy) were used. A 13762 NF mammary adenocarcinoma fragment (100–200 mg) was injected subcutaneously into the flank on day 0 and grew to the estimated size of about 1 g, then the rats were divided randomly into six groups of seven animals each. Two groups were treated with CDDP 1.0 mg/kg twice weekly for 4 weeks and one also received EPO 50 μg/kg ip three times/week; two other groups were treated with CDDP 1.5 mg/kg twice weekly for 4 weeks and one also received EPO as above. Another group was treated with EPO alone (50 μg/kg ip three times/week), and the sixth group remained untreated. An additional group included control untreated rats and two exploratory groups treated with CDDP 2 mg/kg alone or with EPO as above.

2.4. *Evaluation methods*

The animals' general conditions were recorded daily and weight and tumour were measured at the time of CDDP administration. In Experiment 1, at scheduled death, whole blood was obtained from the rats in each group through abdominal aorta puncture and collected in a heparinated tube to chech the haematocrit. Serum from rats treated with CDDP + EPO 50 was tested for anti-human EPO antibodies according to Tacey et al.[25]

2.5. *Haematocrit*

Whole blood was obtained through abdominal aorta puncture and collected in a heparinated tube for the haematocrit, then centrifuged at 2500 rpm for 20 min at 4 °C. Total and erythrocyte capillary tube length was measured and the haematocrit was calculated as a percentage by dividing the particulate by the total length and multiplying it by 100.

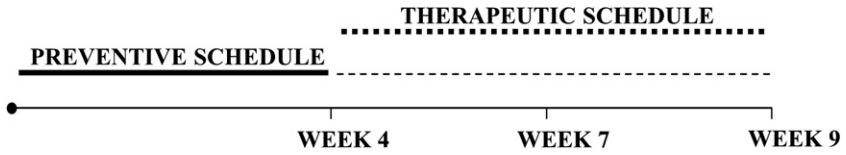

Fig. 1 – Flow-chart of experiment 1 – CDDP 2 mg/kg was injected intraperitoneally twice weekly for eight times. EPO was given twice weekly at 50, 10 and 0.5 μg/kg according to the preventive schedule (thick solid line), then discontinued in order to evaluate the follow-up after 3 and 5 weeks (thin dotted line). In one group of CDDP-treated rats (*n* = 8), a therapeutic schedule with 50 μg/kg EPO twice weekly was started after the last CDDP injection (thick dotted line) and lasted 5 weeks.

2.6. Sensory nerve conduction velocity (SNCV) in the tail nerve

In both experiments, at baseline, at the end of treatment and after the follow-up period when appropriate, SNCV in the tail was measured for each animal by a method already used in experiments on neuroprotection.[19,26] Briefly, the antidromic SNCV in the tail nerve was assessed by placing recording ring electrodes distally in the tail, while the stimulating ring electrodes were placed 5 cm and 10 cm proximally from the recording point. The latencies of the potentials recorded at the two sites after nerve stimulation were determined (peak-to-peak) and nerve conduction velocity was calculated accordingly. All the neurophysiological determinations were done under standard conditions in a temperature-controlled room.

2.7. Tumour growth

The growth of tumour in Experiment 2 was measured twice weekly using a Vernier caliper. The length (L) and width (W) of the tumours were measured and their volumes (TV) were calculated using the formula $(L \times W2)/2$.

The percentage of tumour growth inhibition (TVI %) was determined as: 100 – mean tumour volume of treated group/mean tumour volume of control group × 100. According to the National Cancer Institute standards, a TVI% > 50% was the minimum level for activity; TVI% > 90% was considered a high activity level. T-C was the median time (in days) required for treatment group tumours less the median time (days) for control group to reach a set size (usually 5 g).

2.8. EPOR in 13762 rat mammary carcinoma

2.8.1. Immunohistochemistry

Samples of tumour were formalin-fixed and paraffin-embedded. Five micrometer-thick sections were cut and deparaffinised in xylene then rehydrated in graded alcohols. Slides were steamed in 0.01 mol/L sodium citrate buffer, pH6, in a microwave oven for 15 min. Endogenous peroxidase activity was quenched by 0.3% hydrogen peroxide in methanol for 30 min and specific proteic reactions were blocked by incubation with goat normal serum for 30 min. Slides were then incubated overnight at 4 °C with anti-EPO receptor (*rabbit polyclonal; cloneH-192*, Santa Cruz Biotechnologies, Santa Cruz, CA, USA, 1:300 dilution) antibody. Slides were incubated for 30 min at room temperature with goat anti-rabbit biotinylated IgGs (Vector Laboratories, Burlingame, CA, USA), developed by the ABC method (*Vectastain Elite ABC kit*, Vector Laboratories), revealed with Vector Nova Red (Vector Laboratories) and counter-stained with haematoxylin. For each sample negative controls were also run with omission of the primary antibody or substitution with normal rabbit IgGs. Immunohistochemical stain for EPOR was interpreted by assessing the intensity of staining. Cytoplasmic and/or membrane immunoreactivity was considered positive.

2.8.2. RNA isolation, reverse transcription (RT) and real-time PCR

Total RNA was extracted from the tumour tissue by direct homogenisation in TRIzol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. The RNA concentration was measured spectrophotometrically. Reverse transcription (RT) was carried out at 37 °C for 60 min in 50 μL RT mixture containing 2 μg total RNA, 400 U reverse transcriptase (M-MLV, Invitrogen), 80 U RNase inhibitor (RNaseOUT, Invitrogen), 0.6 mM each of dNTPs (Amersham Biosciences, Piscataway, NJ, USA), 1 μg random primers (Promega, Madison, WI, USA). Aliquots corresponding to 1/25 of the resulting complementary DNA (cDNA) were subjected to real-time PCR, using the TaqMan gene expression assays for rat EPOR and for the housekeeping gene 18S rRNA (Applied Biosystems, Foster City, CA, USA). As a control for contaminating genomic DNA amplification, samples containing RNA which was not reverse transcribed were included in each PCR.

All procedures, including data analysis, were performed on the ABI PRISM 5700 Sequence Detection System (Applied Biosystems, Foster City, California, USA) using the software provided with the instrument. Results were expressed as EPOR mRNA arbitrary units, representing EPOR gene expression in relation to the calibrator sample. We used a cDNA from rat kidney, known to express EPOR as calibrator.

2.9. Thermal nociceptive threshold

The nociceptive threshold to radiant heat was quantified in Experiment 2 using the hot plate paw withdrawal test, as previously described.[15] Briefly, a 40 cm high Plexiglas cylinder was suspended over the hot plate and the temperature was maintained at 50 °C to give a latency of about 10 s for control rats. Paw withdrawal latency was defined as the time between placing the rat on the hot plate and the time of withdrawal, or licking the hind paw, or discomfort manifested by the animal. The test was done every week and animals were tested twice, with a 30 min interval.

2.10. Statistics

The differences in body weight, SNCV and haematocrit were statistically analysed by the analysis of variance (ANOVA) and the Tukey–Kramer post-test.

3. Results

3.1. General observations

In Experiment 1, nine rats died during the treatment phase (five in the CDDP group, two in the CDDP+ EPO 0.5 μg/kg group and two in the CDDP+ EPO 50 μg/kg group), four rats died in the follow-up period (two in the CDDP group, one in the CDDP+ EPO 50 μg/kg ther. group and one in the CDDP+ EPO 10 group) (Fig. 2). CDDP frequently caused pilorection and slightly reduced motility. EPO was well tolerated in the surviving animals and did not appear to induce any obvious side effect.

3.2. Growth rate

No differences in body weight were observed at baseline in Experiment 1 (data not shown). CDDP induced a significant reduction in weight compared to controls at the end of the

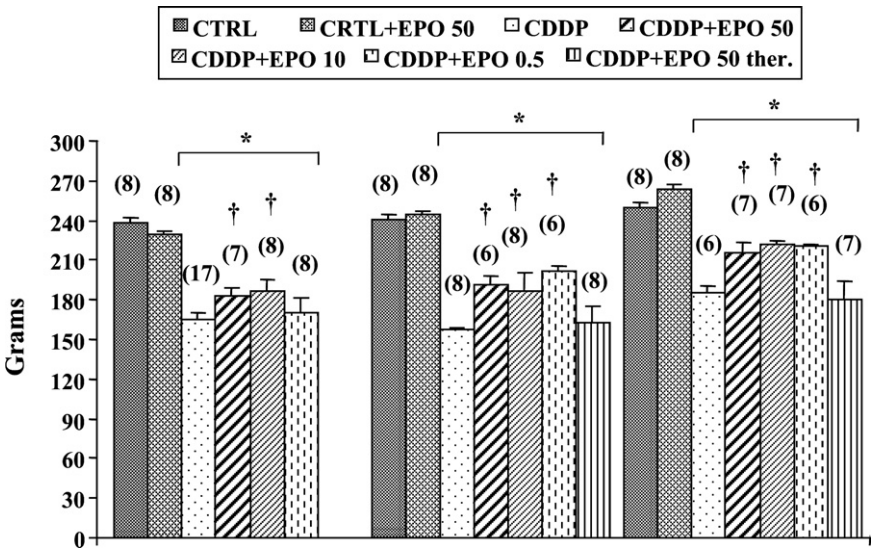

Fig. 2 – Weight of rats treated with CDDP and EPO at different doses according to the experimental design illustrated in Fig. 1 – CDDP reduced the growth rate and EPO at higher doses partially protected against this weight loss (left columns). Body weight recovered in all the CDDP-treated groups at 3 and 5 weeks (central and right columns) after discontinuation of treatment or according to the EPO therapeutic protocol. CRTL: untreated controls; CTRL + EPO 50: EPO-treated controls; CDDP: CDDP-treated rats; CDDP+EPO 50, CDDP+EPO 10, CDDP+EPO 0.5: CDDP-treated plus high, intermediate or low doses of EPO; CDDP+EPO 50 ther.: CDDP, EPO-treated rats starting on day 28. Data are expressed as grams and are the mean ± S.E.M. (number of rats in each group in parentheses). *$P < 0.05$ versus CTRL; †$P < 0.01$ versus CDDP.

experiment (30.7% on day 28; $P < 0.05$ by one-way ANOVA), which was significantly prevented by EPO 50 μg/kg and 10 μg/kg ($P < 0.05$); EPO at the lowest dose was not effective (Fig. 2). Recovery of body weight continued in the follow-up (Fig. 2, central and right panels).

3.3. *Haematocrit*

As expected, EPO at all three dosages significantly raised the haematocrit level administered during CDDP treatment ($P < 0.01$ versus CTRL and CDDP; Fig. 3A), and after CDDP (Fig. 3B). Similarly, when EPO (50 mg/kg) was given on day 28 after CDDP treatments (CDDP + EPO 50 ther.) it significantly ($P < 0.01$) increased the haematocrit (Fig. 3B, last column). In the EPO-treated rats (CDDP + EPO 50) the haematocrit significantly dropped in the follow-up ($P < 0.01$ versus CDDP + EPO on day 28), possibly due to the expected anti-human EPO antibody response in the EPO-treated rats (compare the third columns of Fig. 3A and B). The frankly anaemic rats showed a positive antibody titre: 1:100, while the rats with normal haematocrit were negative for antibodies against EPO (data not shown).

3.4. *Nerve conduction velocity*

The results of the neurophysiological examination in Experiment 1 are reported in Fig. 4. Immediately after treatment (day 28, Fig. 4A), CDDP significantly impaired SNC in the tail nerve by about 30%; the co-administration of EPO 50 (+ 20% $P < 0.01$ versus CDDP) and, to a lesser extent, EPO 10 ($P < 0.05$ versus CDDP) significantly reduced this effect, while low-dose-EPO had no effect on CDDP-induced neurophysiological changes.

Three weeks after treatment withdrawal (Fig. 4B), CDDP+ EPO 50 rats were still significantly less affected than CDDP-treated rats (+ 9% $P < 0.01$); the trend was still positive after 5 weeks but the difference was no longer significant, when (as expected on the basis of the literature regarding this experimental paradigm) CDDP rats made a partial spontaneous recovery (about 20%, Fig. 3B right panel). EPO administered during the follow-up according to the 'therapeutic' schedule (CDDP+ EPO 50 ther. group) induced a significant rise (+39%, $P < 0.01$ versus CDDP, Fig. 3B right panel, last column) but not reaching the control levels (−8.5%, $P < 0.05$ versus CTRL).

3.5. *Expression of EPOR in the mammary tumour*

Immunohistochemistry indicated that a large number of cells were positive for EPOR. Immunoreactivity was prominent in the cellular cytoplasm with a granular appearance, especially distributed at one cellular pole (Fig. 5). EPOR mRNA was expressed in all the tumour areas analysed by real-time RT-PCR (range 0.6–0.9 arbitrary units; data not shown).

3.6. *Tumour growth and EPO effects*

Fig. 6 shows the antitumour activity on 13762 rat mammary carcinoma of CDDP at two doses (1.0 and 1.5 mg/kg twice weekly for 4 weeks) given alone or with EPO. No rats died during treatment, and the controls and EPO-treated groups bearing tumours were killed on day 15 when the tumour reached about 10% of the animal's weight (Fig. 6). EPO did not appear to influence the tumour growth (Fig. 6). CDDP at both doses significantly reduced tumour growth and the co-administration of EPO did not accelerate tumour growth. Table 1

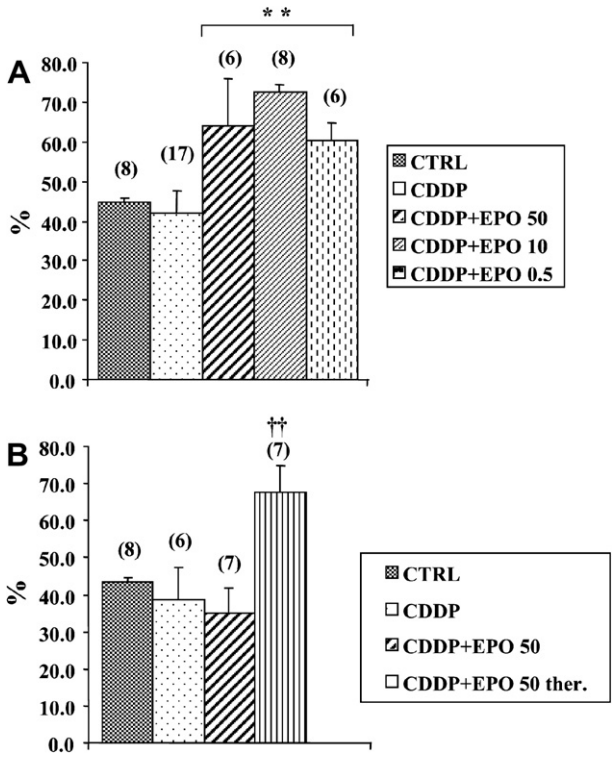

Fig. 3 – Haematocrits according to Experimental design 1 – On day 28, CDDP did not change the haematocrit whereas EPO significantly raised it (Panel A). In the previously EPO-treated rats the haematocrit dropped below the control level in the follow-up (third columns in Fig. 3, panels A and B). In CDDP-treated rats EPO, starting on day 28, raised the haematocrit (Panel B, last column). Legend: see Fig. 2. Data are percentage and are the mean ± S.E.M. (number of rats in each group in parentheses). **P < 0.01 versus CTRL and CDDP; ††P < 0.01 versus CDDP.

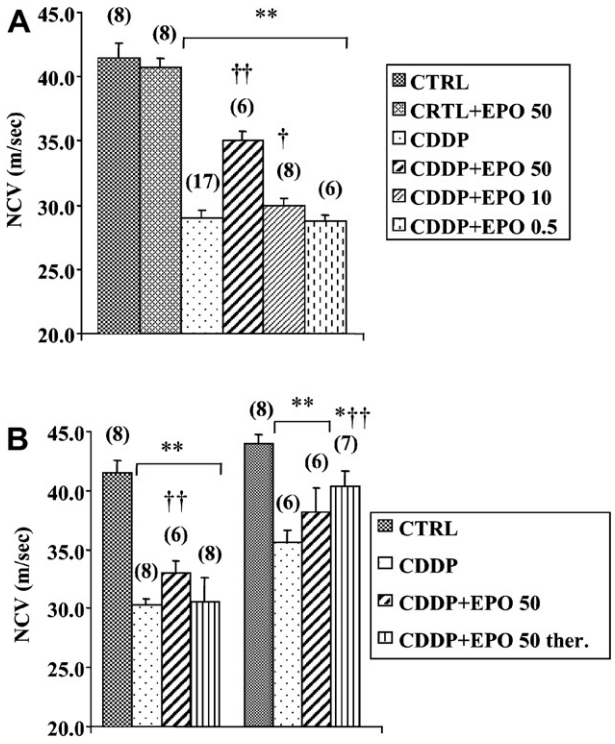

Fig. 4 – Tail nerve conduction velocity in rats in Experiment 1 – At the end of CDDP treatment (day 28, A) and after 3 and 5 weeks follow-up (B, left and right panels) after discontinuation of treatment or according to the EPO therapeutic protocol (B, last column, left and right panels). Data are expressed as m/sec and are the mean ± S.E.M. (number of rats in each group in parentheses). Legend: see Fig. 2. **P < 0.01 versus CTRL and CDDP; ††P < 0.01 versus CDDP.

illustrates the spectrum of anti-tumour efficacy of CDDP alone or with EPO.

The exploratory experiment conducted using CDDP 2 mg/kg showed complete disappearance of the tumour in 80% of rats treated with CDDP alone or the combination of CDDP and EPO (data not shown).

3.7. *Heat sensitivity*

Fig. 7 shows the hind paw heat threshold, before CDDP or EPO and during the study. After CDDP, at both doses, the thermal response latency threshold rose progressively, reaching a maximum at day 21. EPO significantly prevented the increases in thermal nociception threshold in CDDP-treated rats (Fig. 7).

3.8. *Nerve conduction velocity in Fisher rats*

Four weeks after CDDP injections (Fig. 8) the observed reduction in SNCV in CDDP 1.5 treated rats (about 10% $P < 0.01$ versus CTRL) was prevented by EPO ($P < 0.01$ versus CDDP).

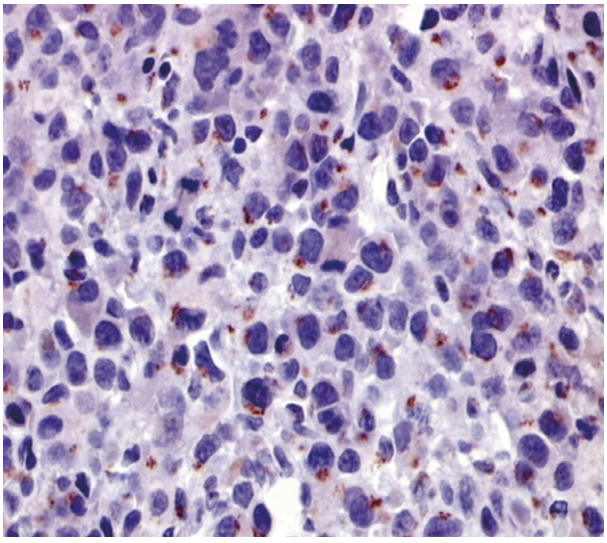

Fig. 5 – Representative microphotographs of immmunochemistry in 13762 mammary tumours, showing moderate granular, cytoplasmic EPOR immunostaining. (Haematoxylin & eosin; original magnification 40×).

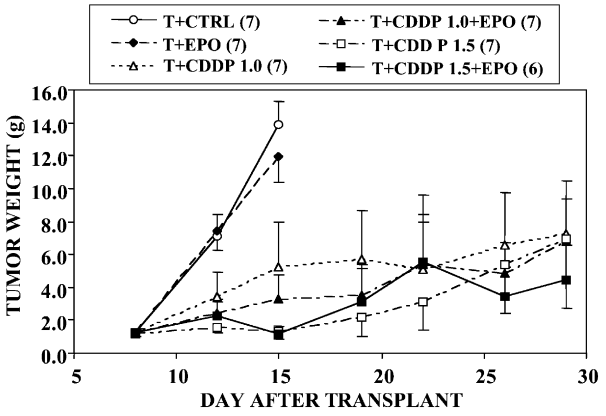

Fig. 6 – Antitumour activity of CDDP alone or with EPO in 13762 mammary carcinoma-bearing rats treated according to Experiment 2 – All rats with tumours were given CDDP at two dosages (1.0 mg/kg or 1.5 mg/kg) and two other groups received CDDP with EPO 50 μg/kg. Tumour weights are shown CDDP at both dosages reduced tumour growth and EPO do not interfere with the antitumoured activity or rat growth. CRTL: untreated controls; CDDP 1.0 mg/kg and CDDP 1.5 mg/kg: CDDP-treated rats at the doses indicated; CDDP 1.0 + EPO and CDDP 1.5+ EPO: CDDP and EPO treated rats at the doses indicated. Data are expressed as grams and are the mean ± S.E.M. (number of rats in each group in parentheses in the box).

Table 1 – Antitumour activity of CDDP alone or in combination with EPO (Experimental design 2)

Group	TVI%[a]	T-C[b] (days)	Tumour-free/ Total rats[c]
CTRL	–	–	0/7
T + EPO	–	–	0/7
T + CDDP 1.0	84	15	3/7
T + CDDP 1.5	94	11	4/7
T + CDDP 1.0 + EPO	90	14	3/7
T + CDDP 1.5 + EPO	91	23	2/6

a Tumour volume inhibition (TVI) on day 19 after tumour implant.
b T-C, tumour growth delay.
c On day 19 after tumour implant.

4. Discussion

EPO, a 165 amino acid sialoglycoprotein which is vital for the regulation of erythropoiesis, is increasingly gaining a role as a neuroprotective agent in models of central and peripheral nervous system diseases.[1,2,4,7,9–11,27,28] In fact, expression of the EPOR in nervous tissue and changes in this receptor in pathological conditions have been demonstrated, supporting the idea that EPO might help prevent or reduce the damage.[3,5,14] Prevention of chemotherapy-induced peripheral neurotoxicity (CINP) with EPO would be particularly interesting, since it is an accepted and safe treatment for anaemia in these patients[4,29,30] and because CINP is an obstacle problem in the current treatment of cancer.[31–36]

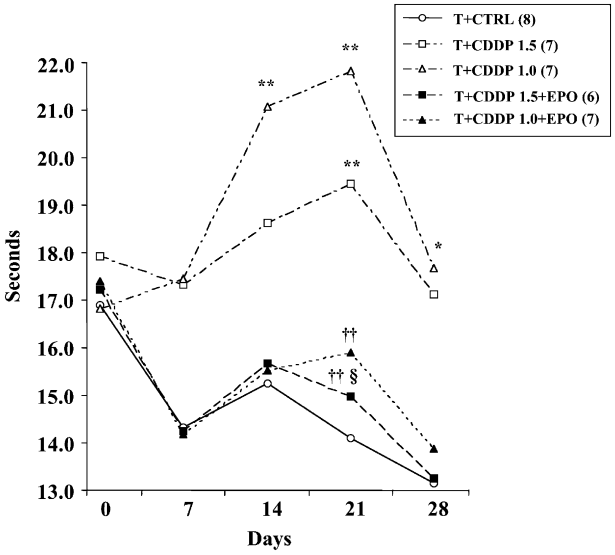

Fig. 7 – Heat sensitivity threshold in CDDP and EPO treated rats in Experiment 2 – EPO prevented changes in the heat threshold in CDDP treated rats. Data are expressed as seconds (number of rats in each group in parentheses in the box). Legend: see Fig. 6. *P < 0.05 versus CTRL; **P < 0.01 versus CTRL; †P < 0.05 versus CDDP 1.0; ††P < 0.01 versus CDDP 1.0; §P < 0.05 versus CDDP 1.5.

Previous studies in our laboratory have shown that EPO can protect and restore nerve function in diabetic neuropathy.[15] Additionally, the use of EPO as a neuroprotectant in CINP has already been demonstrated,[23,24] but no detailed information has been reported on the dose-relationship of its effect. Moreover, although it appears possible to treat the neuropathy once it becomes evident in diabetic neuropathy models, this effect has not yet been investigated in toxic neuropathies. Finally, the question of interference with

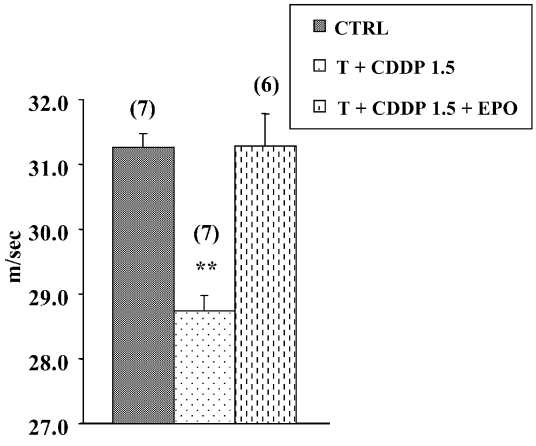

Fig. 8 – Tail nerve conduction velocity in Experiment 2 - At the end of CDDP and EPO treatments on day 28, EPO counteracted the reduction in NCV observed in the CDDP-treated group. Data are expressed as m/sec and are the mean ± S.E.M. (number of rats in each group in parentheses). Legend: see Fig. 6. **P < 0.01 versus CTRL and CDDP.

antineoplastic drugs' action has never been formally investigated in this model.

The model of sensory peripheral neurotoxicity used in the present study is well characterised and has been widely used in pre-clinical studies on CDDP neurotoxicity and neuroprotection.[17–19,21,22,26,37–41] It reproduces the neurophysiological and pathological alterations observed in patients exposed to the drug. Here we show that CINP is associated with growth impairment, reduction in SNCV and an increased thermal threshold. EPO can achieve both protective and therapeutic effects in the setting of CINP in a dose-dependent manner, without enhancing tumour growth. In the range of EPO doses we used there was a clear-cut relationship in neuroprotection, with the best effect at the highest intensity schedule and no effect with the lowest ones.

The mechanism of CINP is still under investigation.[42,43] The peripheral neuropathy developing with repeated administration of CDDP is qualitatively similar to that in humans, involving degeneration of sensory nerve fibres either directly or caused by DRG neuronopathy, or both.[18,44] Long-term observation after CDDP administration to rats, like in patients,[45] showed nerve function improved over time. In addition, like in diabetic neuropathy, the vascular component of peripheral nerves might possibly be involved after CDDP.[46] Here we confirm these observations, and show that EPO administered during the follow-up after CDDP reduced the time to recovery.

As expected, on the basis of previous pre-clinical data,[47,48] our results indicate that EPO in the range 0.5–50 μg/kg (i.e. about 60–6000 IU/kg) markedly raised the haematocrit when co-administered according to our experimental paradigm. In contrast, at the end of the follow-up the haematocrit dropped steeply in rats given EPO 50 μg/kg for 4 weeks during the CDDP treatment, possibly in relation to the occurrence of antibodies against rhEPO, as already noted in 69% of animals, using similar schedules.[47] Titration of antibodies against EPO in these groups of rats confirms this assumption.

In agreement with the neurophysiological benefits, EPO also significantly improved the hind-paw heat threshold in CDDP-treated rats. Since small fibres are deeply involved in thermal and nociceptive pathways, while NCV correlates mostly with the impairment of large myelinated fibres, it seems that EPO is active on both populations, in agreement with findings in neurotoxic models of peripheral neuropathies.[15,24] We have shown that EPO can prevent both the decrease in NCV and the loss of intraepidermal fibre density in CDDP-treated rats.[24]

A possible concern about using EPO to prevent CIPN was whether it interfered with the activity of chemotherapy. Although recent clinical data based on large cohorts of patients and meta-analysis seems to rule this out,[49–51] we investigated this issue using an *in vivo* model of mammary carcinoma expressing EPOR which, therefore, is suitable for assessing this effect of EPO. We already reported that EPO did not interfere with the tissue platinum concentration[24] and here we found no unwanted effect on tumour growth or on the effect of CDDP.

Despite our encouraging results, and confirmed previous observations, the experience with other neuroprotectants in toxic-induced peripheral neuropathy indicates that they should be taken with caution.[52,53]

Overall, these results confirm that EPO is an effective neuroprotectant that does not interfere with platinum-based tumour treatment. The biological action of different doses of EPO on erythropoiesis and on the peripheral nervous system was also demonstrated. This difference calls for further investigation in order to better understand the mechanisms of neuroprotection of EPO and the role of EPO-derived molecules.

Conflict of interest statement

None declared.

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