Cancer Chemother Pharmacol (2009) 64:847–856
DOI 10.1007/s00280-009-1017-6

ORIGINAL ARTICLE

Neuronal expression of copper transporter 1 in rat dorsal root ganglia: association with platinum neurotoxicity

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Received: 8 January 2009 / Accepted: 26 April 2009 / Published online: 24 May 2009

Abstract
Purpose We report the neuronal expression of copper transporter 1 (CTR1) in rat dorsal root ganglia (DRG) and its association with the neurotoxicity of platinum-based drugs.
Methods CTR1 expression was studied by immunohistochemistry and RT-PCR. The toxicity of platinum drugs to CTR1-positive and CTR1-negative neurons was compared in DRG from animals treated with maximum tolerated doses of oxaliplatin (1.85 mg/kg), cisplatin (1 mg/kg) or carboplatin (8 mg/kg) twice weekly for 8 weeks.
Results Abundant CTR1 mRNA was detected in DRG tissue. CTR1 immunoreactivity was associated with plasma membranes and cytoplasmic vesicular structures of a subpopulation (13.6 ± 3.1%) of mainly large-sized (mean cell body area, 1,787 ± 127 μm^2) DRG neurons. After treatment with platinum drugs, the cell bodies of these CTR1-positive neurons became atrophied, with oxaliplatin causing the greatest percentage reduction in the mean cell body area relative to controls (42%; $P < 0.05$), followed by cisplatin (18%; $P < 0.05$) and carboplatin causing the least reduction (3.2%; P = NS). CTR1-negative neurons, with no immunoreactivity or only diffuse cytoplasmic staining, showed less treatment-induced cell body atrophy than CTR1-positive neurons.
Conclusions CTR1 is preferentially expressed by a subset of DRG neurons that are particularly vulnerable to the toxicity of platinum drugs. These findings, together with its neuronal membrane localization, are suggestive of CTR1-related mechanisms of platinum drug neuronal uptake and neurotoxicity.

Keywords Chemotherapy-induced neurotoxicity · Copper transporter · Platinum drug · Dorsal root ganglion · Sensory neuron · Uptake

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Introduction

Chemotherapy-induced peripheral neuropathy is a common and dose-limiting adverse effect of several anticancer drugs. It may limit the delivery of cancer therapy, compromise patient quality of life and persist long beyond the completion of treatment [1]. Chemotherapy-induced peripheral neuropathy occurs in association with particular classes of anticancer drugs, such as platinum and antimicrotubule agents, but individual agents of the same class display differing neurotoxicity profiles. Platinum-based drugs, for example, induce a chronic sensory neuropathy with distal paresthesia and dysesthesiae, loss of deep tendon reflexes, vibration sense and proprioception, and sensory ataxia, coming on or worsening with repeated treatments [2, 3]. However, cisplatin, oxaliplatin and carboplatin differ in their acute neurotoxicity and ototoxicity [4, 5], and in the proportion of patients developing peripheral neuropathy of any severity grade after treatment, which is reported to occur in ~50% [6], ~90% [7] and ~6% [8] of treated patients, respectively. Currently, no

approved treatments are available for preventing or limiting chemotherapy-induced peripheral neuropathy, or managing its symptoms, despite a wide range of neuroprotective therapies having been tested clinically [9].

The mechanism of platinum neurotoxicity remains incompletely understood although it may involve platinum accumulation within the dorsal root ganglia (DRG) leading to atrophy or loss of peripheral sensory neurons. Clinical and electrophysiological features of the sensory neuropathy and the sparing of motor function point to damage occurring at the level of the cell body of sensory neurons within the DRG [10–13]. Histopathological studies have shown altered size profiles of DRG neurons after platinum treatment of patients [11, 14] and in rodent models [15–21], consistent with induction of neuronal atrophy or selective loss of large DRG neurons. High levels of platinum accumulate in the DRG compared to peripheral nerves, spinal cord and brain, following exposure to platinum-based drugs in patients [11, 14, 22] and animal models [15, 21, 23–25], but the differing neurotoxicity profiles of cisplatin, carboplatin and oxaliplatin are not simply explained by differences in DRG platinum concentration [16, 23, 25]. Platinum-based drugs exert their antitumour activity by formation of platinum-DNA adducts, particularly 1,2-intrastrand cross-links between N7 residues of adjacent guanine bases [26]. The cellular mechanism of their neurotoxicity is less clear but platinum-DNA adducts have been detected in DRG neurons [27, 28] and their level correlates with the severity of platinum neurotoxicity [29, 30].

Copper transporter 1 (CTR1) is the major high-affinity copper uptake transporter in mammals and an integral membrane protein containing three transmembrane domains with methionine- and histidine-rich metal binding sites [31, 32]. Evidence implicating CTR1 in the transport of platinum drugs includes demonstration of altered uptake and toxicity of platinum drugs associated with genetic knockout, transfection or copper-induced down-regulation of CTR1 in *Saccharomyces cerevisiae* [33, 34], rodent [33, 35] and human cells [35, 36]. In humans and rodents, CTR1 is expressed by selected normal tissues and cell types [37–39], but it has not been detected before in primary sensory neurons or DRG tissue. However, DRG neurons are known to strongly express several cuproenzymes, such as cytochrome C oxidase [40] and Cu/Zn superoxide dismutase [41], which could indicate significant requirements for copper acquired via CTR1. To investigate the DRG neuronal expression of CTR1, we studied tissues from healthy adult rats by immunohistochemistry and real-time PCR. We then determined the relationship between neuronal CTR1 expression and the neurotoxicity of platinum drugs in animals treated with oxaliplatin, cisplatin and carboplatin.

Materials and methods

Animals and drug treatment

Age-matched, 12-week-old female Wistar rats were housed in a self-contained unit maintained at 22 ± 2°C, and set to 12 h dark–light cycles with food and water ad libitum. Twelve healthy animals were used in immunohistochemistry and PCR studies. In addition, for treatment study, 27 rats were randomly divided into five experimental groups ($n = 5$ or 6) to receive intraperitoneal injections of oxaliplatin (1.85 mg/kg, Sanofi Aventis), cisplatin (1 mg/kg, Sigma), carboplatin (8 mg/kg, Mayne Pharma), dextrose and physiologic saline (Baxter) as vehicle controls, respectively, twice weekly for 8 weeks, at an injection volume of 15 ml/kg given between 1300 and 1500 h. Animals were weighed twice weekly and inspected daily for signs of drug toxicity. All animal procedures were approved by the institutional Animal Ethics Committee (AEC No. R591) and complied with the UKCCCR guidelines throughout.

Reverse-transcriptase PCR and real-time PCR

Healthy animals designated for CTR1 PCR analysis were euthanized by intraperitoneal injection of pentobarbitone (90 mg/kg body weight, Chemstock Animal Health, Christchurch, New Zealand). Tissues from lumbar DRG, cerebral cortex, spinal cord, liver, kidney and duodenum were collected and homogenized in PureZol reagent by a Dounce homogenizer (Glas-Col, Terre Haute, IN, USA) for total RNA isolation using an Aurum Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad, Hercules, CA, USA). Following digestion with DNase I (1 unit/μg, Bio-Rad), total RNA (0.25 μg) of each sample was reverse-transcribed into cDNA using a SuperScript first strand synthesis kit (Invitrogen, Carlsbad, CA, USA) according to instructions, followed by digestion with Ribonuclease H (Invitrogen). cDNA was amplified by PCR in a reaction mixture containing dNTP, $MgCl_2$, Platinum *Taq* DNA polymerase (Invitrogen) and custom primers, using a GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA, USA) at 52°C for 40 cycles. Forward and reverse primers for CTR1 (Genebank accession number NM_133600) were: 5′-ttg gct tta aga atg tgg acc t-3′ and 5′-cat aag gat ggt tcc att tgg t-3′; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (accession number NM_017008): 5′-tgc tga gta tgt cgt gga gtc t-3′ and 5′-aca gtc ttc tga gtg gca gta a-3′, as a control. PCR products were electrophoresed in 2% agarose gel, stained with ethidium bromide and photographed using Gel Doc 2000 System (Bio-Rad). Subsequently, quantitative real-time PCR was performed to compare the relative CTR1 mRNA level in DRG to other tissues using

ABI PRISM 7900HT Sequence Detection Systems (Applied Biosystems). Primers and probe sets were purchased as TaqMan Gene Expression Assays containing forward and reverse unlabeled PCR primer pair and a fluorescent reporter dye-labeled TaqMan MGB probe. Samples containing 25 ng cDNA of each tissue, TaqMan universal PCR Master Mix, FAM-labeled probe for rat CTR1 gene and VIC-labeled probe for 18S ribosomal RNA as endogenous control, were analyzed in triplicate in 10-µl total volume. A total of six rats were used in this study. The abundance of mRNA of CTR1 or rRNA was measured as the threshold cycle values (C_t) after each reaction. The relative RNA expression level of each sample was calculated, with DRG as the arbitrary calibrator for comparison, using the $2^{-\Delta\Delta C_t}$ method [42], where $\Delta\Delta C_t = (C_{t,CTR1} - C_{t,rRNA})_{target\ tissue} - (C_{t,CTR1} - C_{t,rRNA})_{DRG}$.

Immunohistochemistry of dorsal root ganglia

Under terminal anesthesia, transcardiac perfusion was performed with 120 ml each of saline and 4% phosphate buffered paraformaldehyde. Lumbar 5 (L5) DRG were dissected out, post-fixated in the perfusion fixative for 2 h, cryoprotected in 30% sucrose and embedded with Tissue-Tek OCT compound (Sakura Finetechnical, Tokyo, Japan). DRG cryosections of 10-µm mounted onto poly-L-lysine pre-coated slides were processed with 0.2% Triton X-100 in PBS, 1% hydrogen peroxide in 50% methanol, and then the blocking buffer containing 3% normal goat serum and 20 mg/ml bovine serum albumin (ICPbio Ltd, Auckland, New Zealand). A rabbit polyclonal anti-hCTR1 primary antibody (1:500, Novus Biologicals, Littleton, CO, USA) was applied to the DRG sections overnight, followed by incubations with a biotinylated anti-rabbit antibody (1:500, Sigma), an extravidin-peroxidase conjugate (1:500, Sigma), and 0.4 M phosphate buffer containing 0.01% H_2O_2 and 0.5 mg/ml of 3,3′-diaminobenzidine tetrahydrochloride (DAB) (AppliChem, Darmstadt, Germany), respectively. Sections were dehydrated by gradient alcohols, cleared in xylene and coverslipped with DPX mounting medium. Technical negative controls were included by incubating DRG sections with non-immune rabbit IgG1 sera or excluding the primary antibody. Digital images were obtained using an Axiocam digital camera attached to an Axiostar light microscope and analyzed using Axiovision 3.0 software (Carl Zeiss, Hallbergmoos, Germany). For fluorescent double label immunohistochemistry, after elimination of endogenous peroxidase and non-specific blocking, DRG cryosections were incubated in 200 µl Invitrogen Image-iT FX signal enhancer for 30 min. A rabbit anti-hCTR1 antibody (1:1,000, Novus) was added at 4°C for 48 h, followed by the Alexa Fluor 488-labeled anti-rabbit IgG (H + L) (1:500, Invitrogen) at room temperature for 3 h. After wash, sections were incubated with a monoclonal mouse anti-phosphorylated neurofilament heavy subunit (pNF-H) antibody (1:2,000, Swant, Bellinzona, Switzerland) at 4°C for 48 h and the Alexa Fluro 594-labeled anti-mouse IgG (H + L) (1:500, Invitrogen) at room temperature for 3 h. The slides were coverslipped with Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA). Reciprocal omission controls were included to ensure there was no cross-bleeding between the channels. Digital images were acquired using a Leica DMR fluorescence microscope (Leica Microsystems, Wetzler, Germany) with a cooled color Nikon digital camera attached, and analyzed using Nikon EclipseNet and ImageJ software (National Institutes of Health, USA).

Measurement of platinum neurotoxicity by DRG neuronal morphometry

The neurotoxicity of platinum drugs in animals was determined by measuring the mean cell body size and size distribution profile of subpopulations of DRG neurons. CTR1-positive neurons were defined according to the presence of intense plasma membrane and/or punctate cytoplasmic immunoreactivity to CTR1. CTR1-negative neurons were defined as those showing no immunostaining or only light diffuse cytoplasmic immunoreactivity, compared to a negative control. Between 1,847 and 2,635 neurons were analyzed per DRG per animal from evenly spaced tissue sections. The CTR1-positive and CTR1-negative neurons were categorized into three size-based groups: small (<750 µm^2), medium (750–1,750 µm^2) and large (>1,750 µm^2), according to previous studies [18, 43].

Statistics

The differences in mean cell body size, staining frequency and mRNA levels between different groups were assessed by two-tailed *t* test and one-way analysis of variance (ANOVA) with Dunnett's post test using Prism 5.01 software (GraphPad, San Diego, CA), with a P value of <0.05 indicating statistical significance.

Results

Neuronal expression and membrane localization of CTR1 in healthy adult rat DRG

Immunohistochemical analysis was carried out using an affinity-purified polyclonal anti-hCTR1 antibody on rat L5 DRG cryosections. The specificity of the antibody had been previously characterized using a hCTR1-transfected human

ovarian carcinoma A2780 cell line and preabsorption assay with immunizing peptide [37]. Cross-reactivity of the antihuman antibody with murine protein was expected based on 100% sequence homology of the C-terminus in both species [38]. As shown in Fig. 1, there was specific CTR1 immunoreactivity associated with the DRG neuronal cell bodies, but not with their nerve fibers, satellite glial cells or other tissue elements. CTR1 immunohistochemical staining was localized to the plasma membranes of large neuronal cell bodies and/or cytoplasmic vesicular structures in a punctate pattern in a portion of DRG neurons of different sizes. In addition, diffuse lighter cytoplasmic staining with nuclear sparing was present in many of the other DRG neuronal cell bodies.

Double label fluorescence immunohistochemistry of CTR1 and phosphorylated neurofilament heavy subunit (pNF-H), a marker for large DRG neurons and their nerve fibers [44], was carried out with DAPI counterstaining (Fig. 2). The result revealed extensive colocalization of CTR1 with pNF-H cell body staining but not with pNF-H nerve fiber staining or with densely DAPI-stained nuclei of satellite glial cells. This was consistent with the single label immunohistochemistry findings described above showing CTR1 immunoreactivity associated with a subpopulation of mainly large DRG neuronal cell bodies, but not with their nerve fibers, satellite glial cells or other tissue elements of the DRG.

Morphometric analysis of CTR1-positive neurons, defined as those having intense plasma membrane and/or punctate cytoplasmic staining, revealed a subpopulation of DRG neurons with a distinct size profile (Fig. 3). CTR1-positive neurons accounted for 13.6 ± 3.1% of the overall population of neurons of L5 DRG of healthy animals ($n = 6$). The mean cell body size of CTR1-positive neurons was 1,787 ± 127 μm^2 compared to 746 ± 69 μm^2 for the overall population ($P < 0.05$). A greater proportion of CTR1-positive neurons (54.0 ± 12.1%) had large cell bodies (>1,750 μm^2) compared to the overall population (7.0 ± 2.5%) ($P < 0.05$).

We next performed RT-PCR with total RNA purified from different tissues of healthy rats to determine gene expression of CTR1. As shown in Fig. 4, uniformly strong electrophoretic bands were observed in all tissues analyzed, indicative of the abundant expression of CTR1 in lumbar

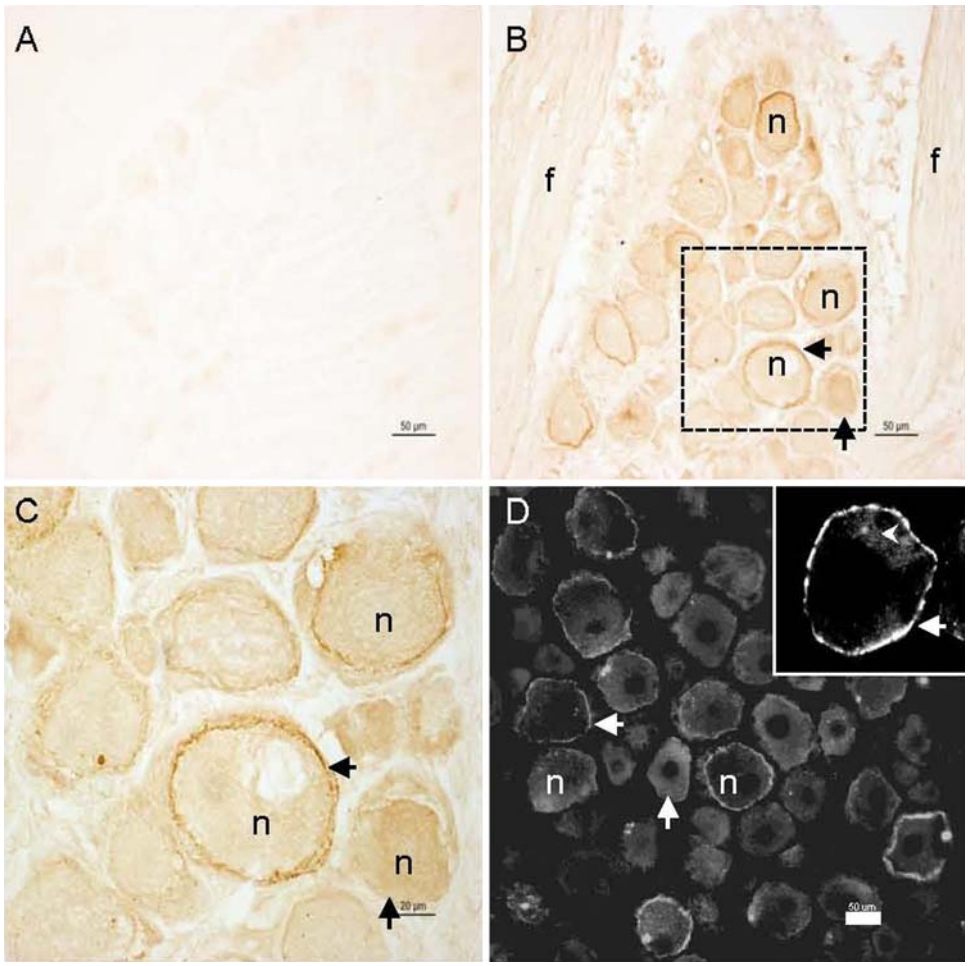

Fig. 1 Neuronal CTR1 immunoreactivity in rat L5 DRG tissue associated with cell bodies (*n*), plasma membranes (←), punctate cytoplasmic vesicular structures (*left arrowhead*) and diffuse cytoplasmic staining (↑) without staining of nerve fibers (*f*) or other tissue elements. **a** negative control, **b** and **c** (enlarged frame in **b**) DAB IHC, **d** fluorescence IHC with higher magnification insert

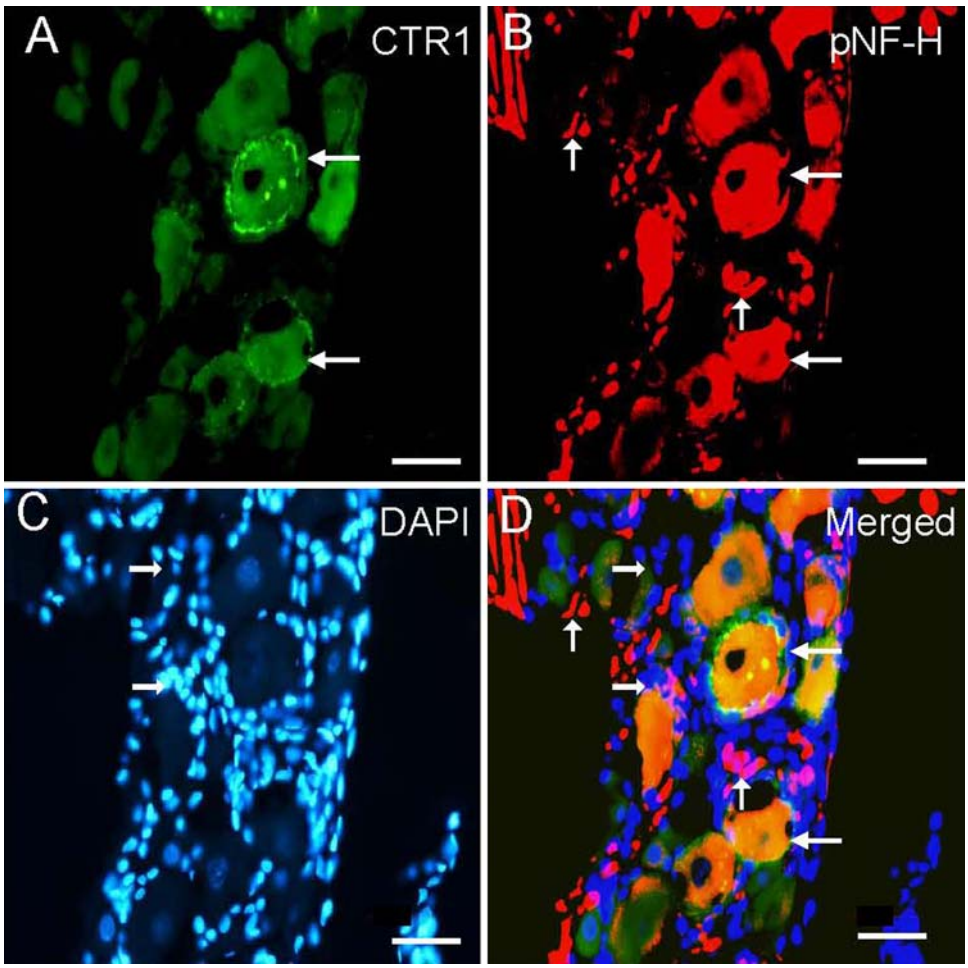

Fig. 2 CTR1 co-localization with phosphorylated neurofilament heavy subunit (pNF-H) cell body staining (←) but not with its nerve fiber staining (↑) or with densely DAPI-stained satellite cells (→). **a** CTR1, **b** pNF-H, **c** DAPI, **d** merged. *Scale bars* 50 μm

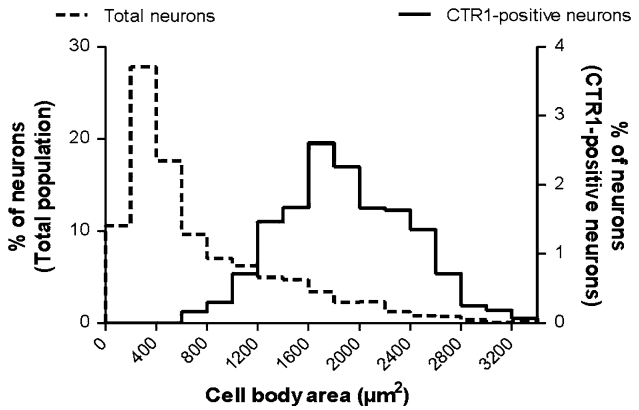

Fig. 3 Cell body size frequency histograms for CTR1-positive DRG neurons (*solid line*) relative to the total DRG neuronal population (*broken line*). Each bin represents the mean value of six animals

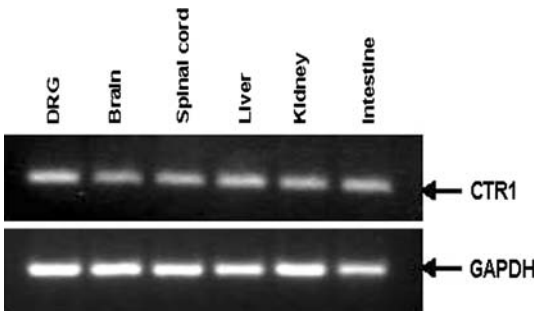

Fig. 4 CTR1 mRNA expression in lumbar DRG and other tissues. Representative gel electrophoretic bands of RT-PCR products for CTR1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in indicated tissues

DRG. These results were further confirmed by quantitative real-time PCR. On average, the relative level of CTR1 mRNA in lumbar DRG appeared similar to brain, spinal cord, kidney, liver and small intestine (0.4- to 3.4-fold, $P \geq 0.05$, ANOVA).

Atrophy of CTR1-positive DRG neurons caused by platinum drug treatment

We examined the association between neuronal expression of CTR1 and the neurotoxicity of a series of clinical platinum drugs in the rat. Use was made of a previously established rat model of chemotherapy-induced peripheral neuropathy and known maximum tolerated doses of

oxaliplatin (1.85 mg/kg), cisplatin (1 mg/kg) and carboplatin (8 mg/kg), with a twice per week for 8 weeks dosing schedule in groups of rats [18, 23]. Peripheral neurotoxicity becomes detectable in this model towards the end of 8 weeks treatment. There was no mortality during the treatment period, but the amount of body weight gained was significantly less in each of the treatment groups compared to their respective control groups (Fig. 5a). Mean percentage change in body weight from baseline during repeated treatment with cisplatin (106 ± 5.5%), carboplatin (105 ± 5.2%) and oxaliplatin (108 ± 2.3%) were similar in each of the treatment groups but less than the dextrose (127 ± 4.8%) or saline (120 ± 3.8%) control groups, suggestive of the different doses of platinum drugs having significant but approximately equivalent general toxicity. One week following completion of all treatment, L5 DRGs were collected, processed immunohistochemically for CTR1 and analyzed morphometrically to determine the frequency of neurons expressing CTR1, and to establish size profiles of CTR1-positive and CTR1-negative neuronal subpopulations in the treatment and control groups. The qualitative pattern of neuronal CTR1 expression in L5 DRG was unchanged by platinum drug treatment. The frequency of CTR1-positive neurons was similar in the dextrose control (12.0 ± 1.2%), saline control (12.5 ± 1.8%), oxaliplatin (12.3 ± 1.8%), cisplatin (12.9 ± 1.1) and carboplatin (13 ± 1.5%) groups ($P > 0.05$, ANOVA).

Treatment of rats with platinum drugs caused atrophy of the cell bodies of CTR1-positive DRG neurons, but the magnitude of their effect varied between the three agents with oxaliplatin causing the greatest toxicity, followed by cisplatin, and carboplatin causing the least. Oxaliplatin was associated with statistically significant reductions in mean cell body size of CTR1-positive neurons (42%, $P < 0.05$, Fig. 5b) and the percentage of CTR1-positive neurons measuring >1,750 μm^2 (86%, $P < 0.05$, Fig. 5c). Oxaliplatin treatment was accompanied by a major leftward deviation of the cell body size frequency histogram for the CTR1-positive neuronal subpopulation (Fig. 6a). In contrast, carboplatin did not cause significant reduction of mean cell body size (3.2%, P = NS, Fig. 5b), the percentage of large neurons (>1,750 μm^2) (35%, P = NS, Fig. 5c) or deviation of the cell body size frequency histogram (Fig. 6a) for the CTR1-positive neuronal subpopulation. The toxicity of cisplatin was intermediate to that of oxaliplatin and carboplatin with statistically significantly reductions in mean cell body area of CTR1-positive neurons (18%, $P < 0.05$, Fig. 5b) and the percentage of CTR1-positive neurons measuring >1,750 μm^2 (35%, $P < 0.05$, Fig. 5c), but these reductions were numerically less than those caused by oxaliplatin. In addition, cisplatin caused a smaller leftward deviation in the cell body size frequency

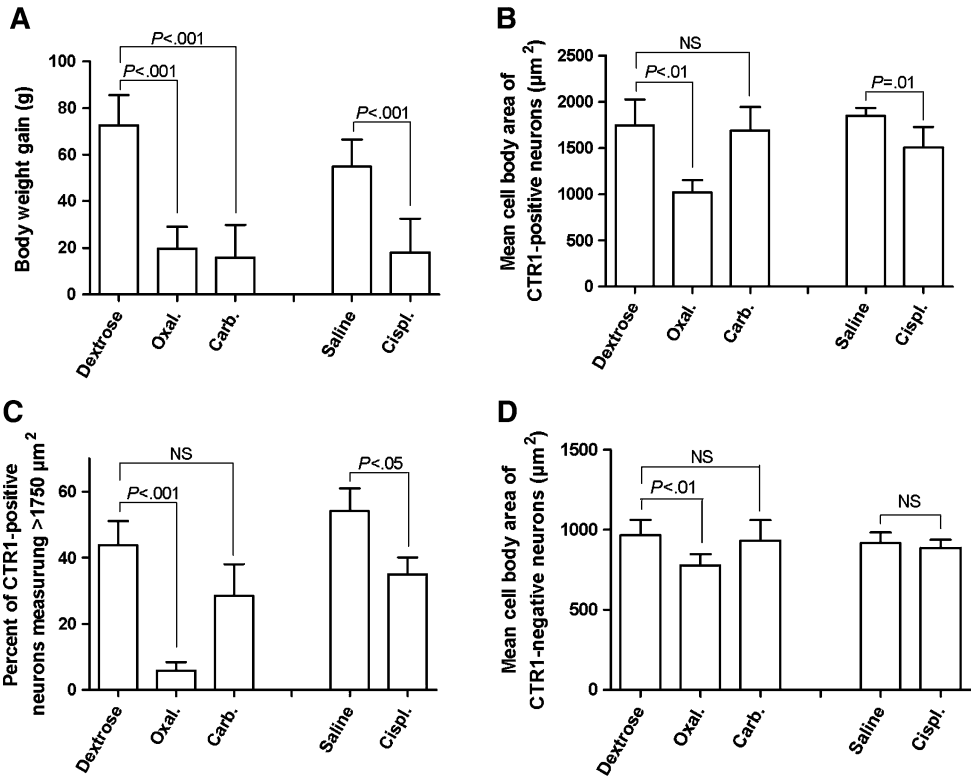

Fig. 5 Effect of oxaliplatin (Oxali.), carboplatin (Carb.) and cisplatin (Cispl.) treatment of animals compared with the vehicle control groups (dextrose, saline). **a** Absolute change in body weight calculated from the body weight of week 8 minus body weight at baseline. **b** Mean cell body size of CTR1-positive DRG neurons. **c** Percentage of CTR1-positive DRG neurons measuring >1,750 μm^2. **d** Mean cell body size of CTR1-negative DRG neurons. Values are expressed as mean ± standard deviation (n = 5 or 6 animals). *NS* not significant

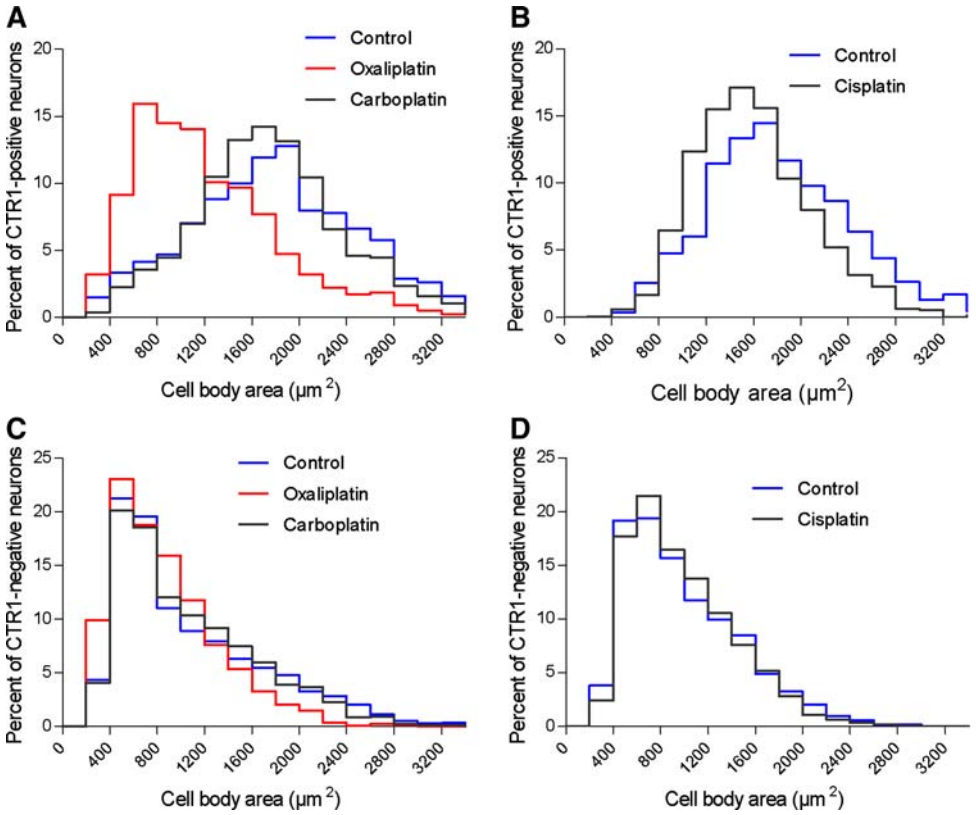

Fig. 6 Comparative effect of oxaliplatin, carboplatin (**a**, **c**) and cisplatin (**b**, **d**) on cell body size frequency histograms of CTR1-positive (**a**, **b**) and CTR1-negative DRG neurons (**c**, **d**)

histogram for the CTR1-positive neuronal subpopulation (Fig. 6b) compared to oxaliplatin.

CTR1-negative DRG neurons were less susceptible to platinum toxicity

The CTR1-negative subpopulation of DRG neurons were altered less by the treatment of rats with platinum drugs than the CTR1-positive subpopulation of neurons. CTR1-negative neurons were defined as those showing no immunostaining or only diffuse cytoplasmic immunoreactivity compared to a negative control. Oxaliplatin significantly reduced the mean cell body area of both subpopulations but the effect on CTR1-positive neurons (42%, $P < 0.05$, Fig. 5b) was numerically greater than that on CTR1-negative neurons (19%, $P < 0.05$; Fig. 5d). Cisplatin significantly reduced the mean cell body area of CTR1-positive neurons versus control (18%, $P < 0.05$, Fig. 5b), but the reduction in the mean cell body area of CTR1-negative neurons was not significant versus control (3.1%, $P =$ NS, Fig. 5d). Furthermore, the cell body size frequency histograms for CTR1-negative neurons (Fig. 6c, d) appeared unchanged or less altered by platinum drugs compared to the clearer leftward deviations in the size profile of CTR1-positive neurons caused by oxaliplatin (Fig. 6a) and cisplatin (Fig. 6b).

Discussion

This study demonstrated the neuronal expression and plasma membrane localization of the copper influx transporter CTR1 in rat DRG. CTR1-immunoreactivity was associated with the cell bodies of DRG neurons but not with their nerve fibers or other tissue elements of the DRG. It was localized to the neuronal surface, with a plasma membrane pattern of immunoreactivity along with staining of cytoplasmic vesicular structures. This pattern of immunoreactivity was seen in a subpopulation of large DRG neurons that accounted for less than 15% of total DRG neurons. These results were further confirmed by the RT-PCR and real-time PCR analysis that indicated abundant CTR1 mRNA level in DRG, comparative to reference tissues. These findings are suggestive of CTR1 having an important but currently uncharacterised physiologic role in subpopulations of primary sensory neurons of the DRG. The localization of CTR1 in the plasma membrane is consistent with it having a role in the uptake of copper into DRG neurons, as in other cell types and tissues [32, 45]. Many neurons categorized as CTR1-negative showed diffuse cytoplasmic immunostaining greater than the negative control but at lower levels than in the CTR-positive neurons. The differential expression of CTR1 may be related to differences in requirements for copper between these two cell types.

Several observations made in the current study suggested significant links between the neuronal expression of CTR1 and the neurotoxicity of platinum-based drugs. For instance, CTR1 expression was demonstrated in a subpopulation of larger-sized DRG neurons that underwent atrophy in response to cisplatin and oxaliplatin treatment, without any change in their number or in the pattern of CTR1 expression. In contrast, the CTR1-negative subpopulation of DRG neurons was smaller, and treatment with oxaliplatin or cisplatin caused less neuronal atrophy, than in the CTR1-positive subpopulation of neurons. Previous studies have suggested that large-sized DRG neurons are more vulnerable to damage from platinum drug treatment than small-sized DRG neurons [18, 19], and that neuronal atrophy could be the morphological basis for the peripheral neurotoxicity that complicates their clinical use [11, 14–20].

In an extension to this finding, we compared the amount of cell body atrophy of CTR1-expressing DRG neurons induced by the treatment of rats with equitoxic doses of cisplatin, oxaliplatin and carboplatin. This quantitative analysis revealed a ranking of these platinum agents according to their effect on the size profiles of CTR1-immunoreactive DRG neurons with oxaliplatin having the greatest effect, followed by cisplatin and then carboplatin. This ranking corresponds with the relative cumulative dose-potencies of oxaliplatin, cisplatin and carboplatin for reducing sensory nerve conduction velocity in rats, which occurs after cumulative doses of 15, 46.7 and 302 μmol/kg, respectively [23]. In addition, this ranking corresponded with the proportion of patients developing peripheral neurotoxicity of any severity grade after treatment with these platinum drugs, which is reported to occur in ~50% [6], ~90% [7] and ~6% [8] of patients treated with cisplatin, oxaliplatin and carboplatin, respectively.

Taken together, these observations implicate CTR1 in the neurotoxicity of platinum-based drugs and suggest a mechanism involving uptake of platinum drugs by CTR1-expressing neurons, leading to neuronal accumulation of platinum and neurotoxicity. A CTR1-based mechanism of neurotoxicity could potentially explain the preferential accumulation of platinum drugs in DRG relative to other tissues [11, 14, 15, 21, 22, 24, 25], and the selective toxicity of platinum drugs to subpopulations of DRG neurons [18, 19]. In addition, differing affinities of platinum drugs for CTR1-mediated uptake [34–36] could contribute to the differing neurotoxicity profiles of individual agents. Alternatively, the mechanism of neurotoxicity could involve disturbance of copper metabolism in CTR1-expressing neurons by platinum drugs, for example via competition for copper uptake [33, 34] or down-regulation of CTR1 protein [46, 47]. The fact that disturbance of copper metabolism has been linked to other neurodegenerative diseases, such as amyotrophic lateral sclerosis [48], strengthens this hypothesis regarding the role of CTR1 in the neurotoxicity of platinum-based drugs. However, until its role is clarified, the possibility remains that it may have no specific mechanistic role in the drug neurotoxicity other than being a marker of the neurons that become damaged by platinum drugs. Higher expression of CTR1 in neurons vulnerable to platinum neurotoxicity may reflect their higher metabolic activity and susceptibility to oxidative damage. In addition, other transporters could be involved in the neurotoxicity, for example those already linked to the transport of platinum-based drugs such as copper transporting P-type ATPases (ATP7A and ATP7B) [49–51], organic cation transporters [52, 53], multidrug resistance proteins [54] and the glutathione S-conjugate efflux pump [55], whose DRG expression is unknown.

In conclusion, CTR1-expressing DRG neurons are particularly vulnerable to the toxicity of platinum drugs, and the extent of their atrophy corresponds with the relative neurotoxicity of oxaliplatin, cisplatin and carboplatin. These findings, together with the neuronal expression and membrane localization of CTR1 in a subset of DRG neurons, are supporting evidence for CTR1-related mechanisms of platinum drug neuronal uptake and neurotoxicity.

Acknowledgments The work was supported by a research grant of Cancer Society of New Zealand.

Conflict of interest statement None.

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