NEUROSCIENCE

Platinum accumulation in the brain and alteration in the central regulation of cardiovascular and respiratory functions in oxaliplatin-treated rats

Ahmed A. Rahman[1,2] · Vanesa Stojanovska[1,3] · Paul Pilowsky[4] · Kulmira Nurgali[1,5,6,7]

Abstract

Oxaliplatin is a platinum-based alkylating chemotherapeutic agent used for cancer treatment. Neurotoxicity is one of its major adverse effects that often demands dose limitation. However, the effects of chronic oxaliplatin on the toxicity of the autonomic nervous system regulating cardiorespiratory function and adaptive reflexes are unknown. Male Sprague Dawley rats were treated with intraperitoneal oxaliplatin (3 mg kg^{-1} per dose) 3 times a week for 14 days. The effects of chronic oxaliplatin treatment on baseline mean arterial pressure (MAP); heart rate (HR); splanchnic sympathetic nerve activity (sSNA); phrenic nerve activity (PNA) and its amplitude (PNamp) and frequency (PNf); and sympathetic reflexes were investigated in anaesthetised, vagotomised and artificially ventilated rats. The same parameters were evaluated after acute oxaliplatin injection, and in the chronic treatment group following a single dose of oxaliplatin. The amount of platinum in the brain was determined with atomic absorption spectrophotometry. Chronic oxaliplatin treatment significantly increased MAP, sSNA and PNf and decreased HR and PNamp, while acute oxaliplatin had no effects. Platinum was accumulated in the brain after chronic oxaliplatin treatment. In the chronic oxaliplatin treatment group, further administration of a single dose of oxaliplatin increased MAP and sSNA. The baroreceptor sensitivity and somatosympathetic reflex were attenuated at rest while the sympathoexcitatory response to hypercapnia was increased in the chronic treatment group. This is the first study to reveal oxaliplatin-induced alterations in the central regulation of cardiovascular and respiratory functions as well as reflexes that may lead to hypertension and breathing disorders which may be mediated via accumulated platinum in the brain.

✉ Kulmira Nurgali
  kulmira.nurgali@vu.edu.au

1 College of Health and Biomedicine, Institute for Health and Sport, Victoria University, Melbourne, Australia

2 Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia

3 Hudson Institute of Medical Research, Monash Health Translation Precinct, Melbourne, Australia

4 Heart Research Institute, Central Clinical School, The University of Sydney, Sydney, Australia

5 Department of Medicine Western Health, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia

6 Regenerative Medicine and Stem Cells Program, Australian Institute of Musculoskeletal Science (AIMSS), Melbourne, Australia

7 Enteric Neuropathy Lab, Western Centre for Health, Research & Education, Sunshine Hospital, 176 Furlong Road, St Albans, Melbourne, Victoria 3021, Australia

Abbreviations

AAS	Atomic absorption spectrophotometry
ANS	Autonomic nervous system
BP	Blood pressure
DRG	Dorsal root ganglia
HR	Heart rate
i.p.	Intraperitoneal
i.v.	Intravenous
MAP	Mean arterial pressure
NO	Nitric oxide
PE	Phenylephrine hydrochloride
PNA	Phrenic nerve activity
PNamp	Phrenic nerve amplitude
PNf	Phrenic nerve frequency
SNP	Sodium nitroprusside
sSNA	Splanchnic sympathetic nerve activity

Introduction

Oxaliplatin (*trans*-l-diaminocyclohexane oxaliplatinum) is a first-line chemotherapeutic agent used alone or in combination, for the treatment of various cancers including metastatic colorectal cancer [19, 37]. It is a third-generation platinum-based alkylating agent with a 1,2-diaminocyclohexane carrier ligand. Like other platinum derivatives, oxaliplatin shows biological activity after being transformed to diaminocyclohexane platinum (Pt(DACH)) derivatives Pt(DACH)Cl$_2$ in the biological system [39]. However, oxaliplatin is preferred to other platinum-based chemotherapeutic agents because it lacks nephrotoxicity unlike cisplatin and due to low hematologic toxicity compared to carboplatin [11, 33]. Platinum accumulates in the brain, spinal cord and periphery (dorsal root ganglia (DRG) and sciatic nerve) following oxaliplatin treatment.

The major dose-limiting adverse effect of oxaliplatin treatment is neurotoxicity leading to peripheral neuropathy [14], which often demands treatment cessation. Dose reduction compromises the efficacy of anti-cancer treatment putting a significant negative impact on the quality of life of most cancer patients [6]. Oxaliplatin causes apoptosis of sensory DRG neurons [45] participating in somatosensory transduction. Accumulation of platinum from oxaliplatin and its binding with neuronal DNA may cause the apoptosis of the DRG neurons [4, 23]. Moreover, platinum-based drugs cause severe cardiovascular toxicity [13, 47], which was attributed to alterations in the function of the autonomic nervous system (ANS) [6, 43]. However, these studies measured changes only in cardiac rhythm and blood pressure (BP) in response to posture change. Failure of baroreflex function also results in cardiovascular toxicity [21, 29]. Anti-cancer chemotherapy appears to alter the baroreceptor reflex [2] that may take part in the cardiovascular mortality of chemotherapy patients. However, the exact mechanism is not clearly understood.

Oxaliplatin is a safer drug compared to other platinum-based chemotherapeutic agents, but its neurotoxic potential to impair cardiovascular and respiratory functions is unknown. To date, no study reveals the direct effect of oxaliplatin treatment on autonomic and phrenic nerve activity that is essential for the moment-to-moment control of cardiorespiratory functions. The effects of oxaliplatin on the sympathetic reflexes, which help to maintain cardiorespiratory parameters at constant levels, have not been studied. We hypothesise that oxaliplatin penetrates the brain and modulates cardiorespiratory functions and sympathetic reflexes by altering the sympathetic and parasympathetic nervous system. We aimed to determine (i) if basal physiological functions and cardiorespiratory reflexes were altered following chronic oxaliplatin treatment; (ii) the effects of acute oxaliplatin injection on cardiorespiratory functions and reflexes in vehicle-treated animals; (iii) if the parameters observed in aim (i) were altered with further systemic injection of a single dose of oxaliplatin; and (iv) platinum accumulation in the brain after chronic oxaliplatin treatment. Physiological functions that were observed include BP, HR, splanchnic sympathetic nerve activity (sSNA), and phrenic nerve activity (PNA). The cardiorespiratory reflexes induced and analysed were baroreceptor reflex, somatosympathetic reflex and central chemoreceptor reflex.

Methods

Animals

All animal experiments in this study complied with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Victoria University Animal Ethics Committee (AEETH_12-018). Male Sprague Dawley rats ($n = 26$, 350–430 g) were obtained from Animal Resources Centre (Perth, WA, Australia). All rats were housed in a temperature-controlled environment with 12-h day/night cycles at the animal holding room at the Western Center for Health, Research and Education (Melbourne, Australia). Standard laboratory rat chow and tap water were available ad libitum. The rats were allowed to acclimatize for at least 7 days before experimental manipulations. Food and water were withdrawn 30 min before anaesthesia.

Treatments

The rats were randomly divided into two groups: treatment group ($n = 13$) and vehicle-treated group ($n = 13$). In the treatment group, the rats received intraperitoneal (i.p.) injections of oxaliplatin (3 mg kg^{-1} per dose) 3 times a week for 14 days (chronic treatment) via a 26-gauge needle. The maximum volume was 400 μL per injection. The oxaliplatin dose was calculated to be equivalent to the standard human dose per body surface area [35, 36]. In the vehicle-treated group, the rats received i.p. injections of sterile water 3 times a week for 14 days via a 26-gauge needle. A single dose of oxaliplatin (3 mg kg^{-1}, 300–400 μL) was injected systemically (intravenously, i.v.) following surgery in the treatment group to determine any further change in the observed parameters.

Electrophysiological experiments

Experimental design

Electrophysiological experiments were conducted as described previously [30, 31]. Briefly, rats were anaesthetised with urethane (1.2–1.4 g kg^{-1}, i.p.). Supplemental doses of urethane (30–40 mg, i.v.) were given when necessary if

nociceptive stimuli (tested every half an hour) caused a change in MAP of more than 10 mmHg. Rectal temperature was maintained at ~ 36.5 °C with a thermostatically controlled heating pad (Harvard Apparatus, Holliston, MA, USA) and infrared heat lamp.

The left jugular vein and right carotid artery were cannulated with polyethylene tubing (internal diameter = 0.58 mm; outer diameter = 0.96 mm) for administration of drugs and fluids, and for the measurement of BP. In some experiments, both femoral veins were cannulated to enable administration of sodium nitroprusside (SNP) or phenylephrine hydrochloride (PE). The trachea was cannulated to enable artificial ventilation, and a 3-lead electrocardiogram was fitted. HR was derived from the BP. The left greater splanchnic and phrenic nerves were isolated, tied with silk thread and cut distally to permit recording of efferent sSNA and PNA respectively. In a subset of animals, the sciatic nerve was isolated, tied and cut distally for electrical stimulation to allow activation of the somatosympathetic reflex. The rats were secured in a stereotaxic frame, vagotomised, paralysed (pancuronium bromide; 0.8 mg initially, then 0.4 mg h^{-1}) and artificially ventilated with oxygen-enriched room air. The animals were infused with 5% glucose in water (1.0–2.0 mL h^{-1}) to ensure hydration. Nerve recordings were made with bipolar silver wire electrodes. The neurograms were amplified (× 10,000, CWE Inc., Ardmore, PA, USA), bandpass filtered (0.1–2 kHz), sampled at 3 kHz (1401 plus, CED Ltd., Cambridge, UK) and recorded on the computer using Spike2 software (v7, CED Ltd.).

Cardiorespiratory reflexes were evoked as described previously [1, 32, 42], with sequential i.v. injections of SNP (10 mg kg^{-1}) and PE (10 mg kg^{-1}) to induce baroreflex or electrical stimulation (10–25 V; 0.2 ms pulse width; 50 sweeps at 1 Hz) of the sciatic nerve to induce the somatosympathetic reflex. Chemoreceptors were activated by ventilating the animals with 5% CO_2:95% O_2 (3 min, hypercapnia; targeting central chemoreceptors; BOC gas and gear, VIC, Australia). Reflexes were activated before and after i.v. injection of vehicle (sterile water, 300–400 μL) or oxaliplatin (3 mg kg^{-1}, 300–400 μL) in each group. The rats were killed with i.v. injection of KCl (3 M, 0.5 mL).

Data acquisition

Neurograms were rectified and smoothed (sSNA, 1 s time constant; PNA, 50 ms). Minimum background activity after death was taken as zero sSNA, and this value was subtracted from sSNA before analysis with off-line software (Spike 2 version 7, CED Ltd., Cambridge, UK). Baseline values were obtained by averaging 60 s of data 5 min prior to oxaliplatin or vehicle injection, and maximum changes were expressed as absolute (MAP, HR, phrenic nerve frequency (PNf)) or percentage (sSNA and phrenic nerve amplitude (PNamp))

changes from baseline values. sSNA was rectified and smoothed at 1 s and 5 ms time constants to analyse the baroreceptor reflex and somatosympathetic reflex respectively. To analyse reflexes, sSNA was normalised between the activity of sSNA before vehicle injection (100%) and the sSNA after death (0%). The sSNA response to sciatic nerve stimulation was analysed using peristimulus waveform averaging. The percentage of peak heights of the sympathoexcitatory peaks was analysed. The response to hypercapnia (5% CO_2:95% O_2 inhalation for 3 min) was quantified by comparing the average maximum sSNA during hypercapnia compared with a control period during normal hyperoxic ventilation. The maximum response to stimulation was then expressed as a percentage change from the baseline (control) considering maximum reflex response in control as 100%.

To analyse the data from the baroreflex function tests, mean MAP was divided into 1-s consecutive bins, and the average sSNA during each bin was determined; successive values were tabulated and graphed as XY plots, taking MAP as the abscissa and sSNA as the ordinate. Each data set was then analysed to determine the sigmoidal curve of best fit [20], which is described by

$$y = A1/[1 + \exp\{A2(x-A3)\}] + A4 \qquad (1)$$

where y is sSNA, x is MAP, $A1$ is the y range (y at the upper plateau–y at the lower plateau), $A2$ is the gain coefficient, $A3$ is the value of x at the midpoint (which is also the point of maximum gain) and $A4$ is y at the lower plateau. The computed baroreflex function curves were differentiated to determine the gain of sSNA of the baroreflex across the full range of MAP, and the peak gain of each curve was determined. The range of sSNA was calculated as the difference between the values at the upper and lower plateaus of the curve. The threshold and saturation values for MAP were defined as the values of MAP at which y was 5% (of the y range) below and above the upper and lower plateaus, respectively [24].

Platinum analysis by atomic absorption spectrophotometry

To determine platinum accumulation, the atomic absorption spectrophotometry (AAS) technique was employed, as previously published [44]. Rat brains were collected; the brainstem and cerebrum were manually homogenised using a Potter-Elvehjem PTFE pestle and glass mortar. Once the samples were prepared, they were diluted to a volume of 4 mL to allow an adequate sample volume for analysis of platinum concentrations. Samples were then aspirated into a Shimadzu AA-6300 AAS (Tokyo, Japan). The specific AAS conditions used to carry out these analyses for platinum included an air-acetylene flame, with a fuel flow of 1.5 L/min and an air flow 15 L/min. The burner height was optimised, and the analytical

wavelength used was 265.9 nm. Background correction was supplied by a D2 lamp, and a slit width of 0.7 nm was used. The lamp current used for platinum was 25 mA. Standard calibration curves were produced before running the samples, with concentration ranges of 10–40 ppm being utilised. Samples were aspirated, with 3 repeat measurements recorded following an initial 2 s pre-spray time. Individual measurements were taken by averaging the absorbance readings over 3 s, which also allowed the calculation of a relative mean square percentage uncertainty. These three measurements were then averaged to give a final absorbance reading for each sample. Concentration values for the unknown samples were calculated automatically by the Shimadzu AAWizard software (Tokyo, Japan).

Data analysis

Grouped data are expressed as mean ± SEM. Statistical analysis was conducted with GraphPad Prism (version 7.0) (Graph-Pad, La Jolla, CA, USA). Unpaired t test was used to analyse peak effects and reflexes. $P < 0.05$ was considered significant.
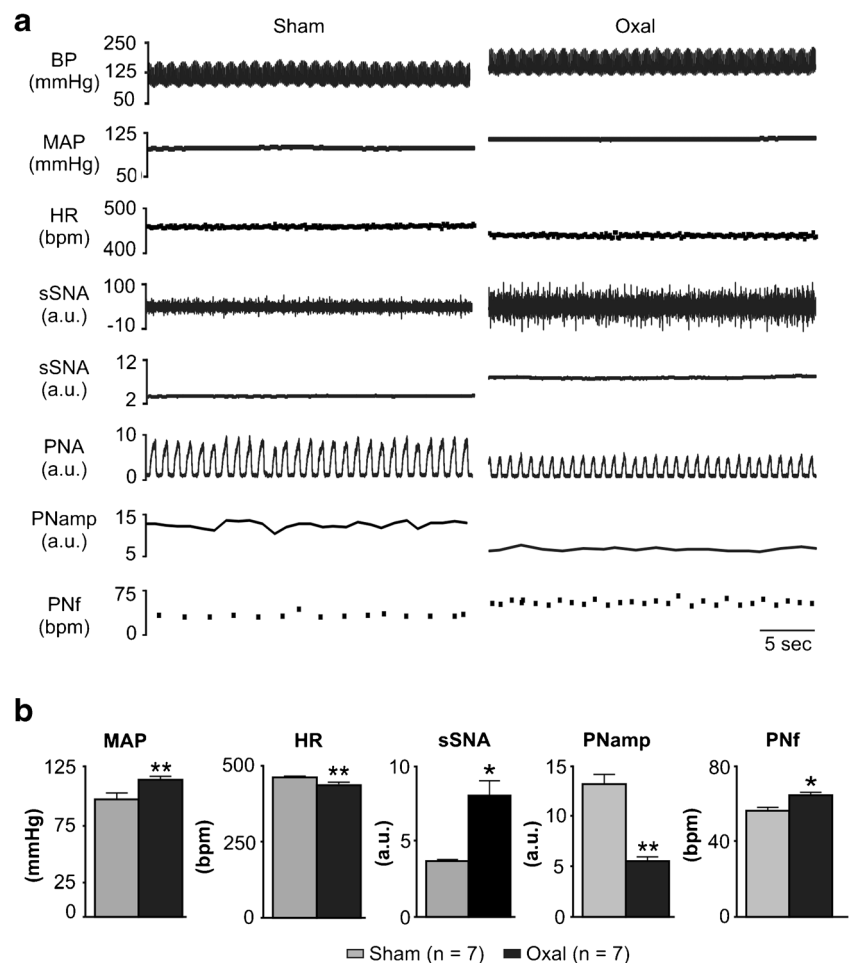
Drugs

Oxaliplatin (MW:397.29; Cat. No: RDS262350-50MG) was obtained from Tocris Bioscience (UK). Urethane, glucose, PE and SNP were purchased from Sigma-Aldrich (Castle Hill, Australia), and pancuronium bromide was obtained from AstraZeneca Pty Ltd. (Sydney, Australia). Oxaliplatin was dissolved in sterile water in order to make 10^{-2} M L^{-1} stock solution and kept frozen at -20 °C. The stock was then defrosted and further diluted with sterile water to make 10^{-3} M L^{-1} solution for injections. PE and SNP were prepared in de-ionised water. Urethane was dissolved in 0.9% NaCl.

Results

Effects of chronic oxaliplatin treatment on cardiorespiratory parameters at rest

Changes in the baseline cardiorespiratory parameters were evaluated to determine any shift following chronic oxaliplatin treatment (Fig. 1). Baseline MAP (113 ± 2.9 mmHg vs $97 \pm$

Fig. 1 Effect of chronic oxaliplatin (Oxal) treatment on cardiorespiratory parameters. **a** Representative recording of blood pressure (BP) (pulsatile and mean), heart rate (HR), splanchnic sympathetic nerve activity (sSNA) and phrenic nerve activity (PNA) (rectified) [arbitrary units (a.u.)], phrenic nerve frequency (PNf) and phrenic nerve amplitude (PNamp) in vehicle-treated and chronic oxaliplatin-treated rats. **b** Grouped data of baseline cardiovascular and respiratory effects in vehicle-treated and oxaliplatin-treated rats. Effects are shown as absolute (mean arterial pressure (MAP), HR (bpm, beats per minute), PNf (bpm, bursts per minute)) or arbitrary (sSNA, PNamp) values. Data are expressed as mean ± SE. Note that chronic Oxal treatment causes a greater increase in MAP, sSNA and PNf while significantly reducing HR and PNamp compared to the vehicle-treated group. The number of animals is shown in parentheses. **$P < 0.01$, *$P < 0.05$ compared to the vehicle-treated group

5.3 mmHg, $P < 0.01$) and sSNA (8 ± 1.0 a.u. vs 3.7 ± 0.1 a.u., $P < 0.05$) were significantly increased in chronic oxaliplatin-treated rats ($n = 7$) as compared to the vehicle-treated group ($n = 7$), whereas baseline HR was significantly decreased (438 ± 6.7 bpm vs 459 ± 3.9 bpm, $P < 0.01$) (Fig. 1).

Chronic oxaliplatin treatment ($n = 7$) also caused a significant increase in the baseline PNf (64 ± 1.5 bpm vs 56 ± 1.9 bpm, $P < 0.05$) value but a reduced baseline PNamp (5.5 ± 0.4 a.u. vs 13.2 ± 1.0 a.u., $P < 0.01$) value as compared to the vehicle treatment ($n = 7$, Fig. 1).

Effects of chronic oxaliplatin treatment on the baroreceptor reflex

In the baroreceptor reflex, the changes in sSNA were plotted against the changes in MAP evoked by i.v. injections of SNP and PE in the anaesthetised rats. At baseline, the treatment group showed significant potentiation in the upper plateau, range of sSNA and operating range as compared with the vehicle-treated group ($n = 7$/group, Fig. 2 and Table 1). But the maximum gain of the sSNA and the threshold level of MAP were significantly reduced in the treatment group without significant
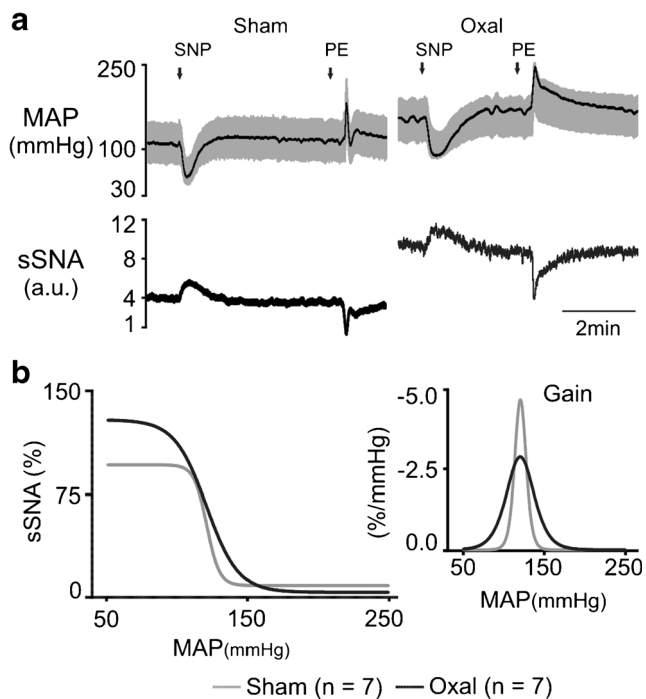

Fig. 2 Effect of chronic oxaliplatin (Oxal) treatment on the arterial baroreflex evoked by intravenous injection of sodium nitroprusside (SNP) and phenylephrine hydrochloride (PE). **a** Representative experimental recording of the effect of changes in blood pressure (BP) on splanchnic sympathetic nerve activity (sSNA) due to SNP or PE in vehicle-treated and chronic oxaliplatin-treated rats. **b** Average splanchnic sympathetic baroreflex function curves generated for data at baseline in vehicle-treated and oxaliplatin-treated rats. The trace on the right represents baroreflex gain for sSNA (error bars are omitted for clarity—see Table 1). In Oxal-treated rats, there is a significant decrease in maximum gain compared to vehicle-treated rats

Table 1 Parameters describing baroreflex control of sSNA at baseline in sham and oxaliplatin-treated rats

	Lower plateau (%)	Upper plateau (%)	Mid point (mmHg)	Max. gain (%/mmHg)	Gain coefficient	Range of sSNA (%)	Threshold level (mmHg)	Saturation level (mmHg)	Operating range (mmHg)
Sham	9.3 ± 1.4	99.9 ± 5.2	126.5 ± 4.3	-4.8 ± 0.6	0.19 ± 0.06	90.6 ± 3.9	119.7 ± 4.6	145.2 ± 10.0	26.9 ± 3.3
Oxaliplatin	12.0 ± 2.3 (ns)	122.1 ± 5.2 ($P < 0.05$)	141.9 ± 5.1 (ns)	-2.7 ± 0.4 ($P < 0.05$)	0.07 ± 0.01 (ns)	127.2 ± 8.9 ($P < 0.05$)	86.4 ± 2.4 ($P < 0.01$)	152.9 ± 4.3 (ns)	82.8 ± 10.7 ($P < 0.01$)

Values are means $\pm$ SEM ($n = 6$). Maximum (Max.) gain is the slope of the sigmoid curve of best fit at the MAP corresponding to the steepest part of the curve

ns non-significant

alteration in the lower plateau, gain coefficient, midpoint and the saturation levels of MAP as compared with the vehicle-treated group ($n = 7$/group, Fig. 2 and Table 1).

Effects of chronic oxaliplatin treatment on somatosympathetic and central chemoreceptor reflexes

Intermittent (discontinuous) stimulation (at three times threshold to elicit a minimal excitatory response) of the sciatic nerve resulted in two characteristic excitatory peaks in sSNA with latencies of 78 ± 1 ms and 167 ± 5 ms at rest in vehicle-treated rats ($n = 7$, Fig. 3a). These two excitatory peaks result from the activation of both A- and C-fibre nociceptors following sciatic nerve stimulation. The latencies of the peaks were significantly longer at rest in chronic oxaliplatin-treated rats (88 ± 3 ms and 179 ± 3 ms, $n = 7$, $P < 0.05$, Fig. 3b). Both first and second excitatory peaks were significantly reduced by 40% and 30% of baseline, respectively, in the treatment group as compared to the vehicle-treated group ($P < 0.05$; $n = 7$/group, Fig. 3b).

Activation of central chemoreceptors with hypercapnia evoked an increase in MAP, HR and sSNA in both vehicle-treated and oxaliplatin-treated rats (Fig. 3c). These changes were enhanced following activation of the central chemoreflex in chronic oxaliplatin-treated rats. The increases in HR (6 ± 0.5 bpm vs 3 ± 0.3 bpm, $P < 0.01$) and sSNA ($25.7 \pm 2.8\%$ vs $13.9 \pm 2.7\%$, $P < 0.05$) following hypercapnia were significantly higher in the treatment group without any significant alteration in MAP response (14 ± 4.2 mmHg vs 8 ± 2.3 mmHg, ns) as compared to the vehicle-treated group ($n = 7$/group, Fig. 3d).

Effects of a single dose of oxaliplatin injection in the vehicle-treated group

Effects of acute oxaliplatin injection on cardiorespiratory parameters

In the vehicle-treated group, acute injection of oxaliplatin (3 mg kg^{-1}) caused no changes in MAP, HR or sSNA as
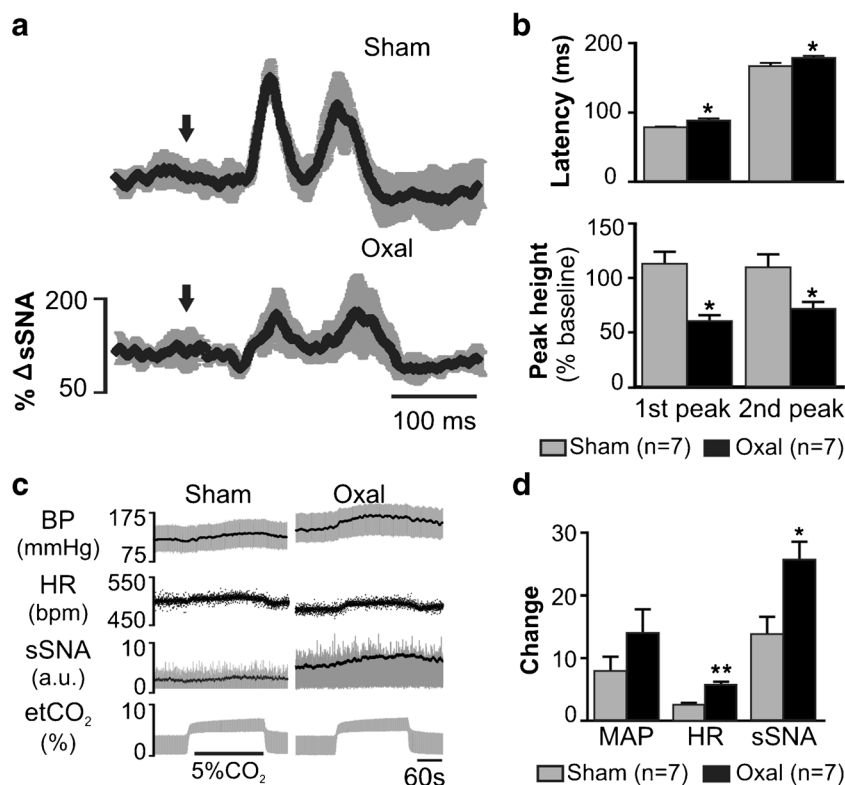

Fig. 3 Effect of chronic oxaliplatin (Oxal) treatment on somatosympathetic and central chemoreceptor reflexes. **a** Grouped effect of sciatic nerve-evoked stimulation of splanchnic sympathetic nerve activity (sSNA) at baseline in vehicle-treated and chronic oxaliplatin-treated rats. Data are mean (black) ± SE (grey). Arrows indicate the time of stimulation. **b** Grouped data illustrating the changes in latency and the 1st and 2nd sympathoexcitatory peaks in oxaliplatin-treated rats compared to vehicle-treated rats. Note that chronic Oxal treatment causes increased latency, but a marked reduction of sympathoexcitatory peaks

compared to vehicle treatment. **c** Experimental recording of hypercapnic episodes with 5% CO$_2$ in oxygen for 3 min in vehicle-treated and chronic oxaliplatin-treated rats. **d** Grouped data of peak effects on cardiovascular (mean arterial pressure (MAP), heart rate (HR: bpm, beats per minute)) and splanchnic sympathetic nerve activity (sSNA) parameters in response to hypercapnia in vehicle-treated and oxaliplatin-treated rats. Following chronic Oxal treatment, there is a greater increase in pressor and symapathoexcitatory response after hypercapnia compared to vehicle treatment. Values are expressed as mean ± SE. **$P < 0.01$, *$P < 0.05$

compared to vehicle injection ($n = 6$) (data not shown). Similarly, the respiratory parameters, i.e. PNf and PNamp, were not changed significantly following acute oxaliplatin administration compared to the vehicle (data not shown).

Effects of acute oxaliplatin injection on baroreceptor, somatosympathetic and central chemoreceptor reflexes

In the vehicle-treated group, acute injection of oxaliplatin significantly increased only the upper plateau without significant alteration in any other parameters of the baroreflex curve as compared to vehicle injection ($n = 6$; Fig. 4a and Table 2).

In the somatosympathetic reflex, the latencies of the first and second excitatory peaks were not significantly altered following injection of acute oxaliplatin (80 ± 1 ms and 169 ± 4 ms, $n = 6$, ns) or vehicle (82 ± 3 ms and 173 ± 5 ms, $n = 6$, ns) as compared to baseline (78 ± 1 ms and 167 ± 5 ms). Acute administration of oxaliplatin also caused no changes in the height of both the excitatory peaks of the somatosympathetic reflex compared to baseline ($n = 6$, Fig. 4b) in vehicle-treated rats.
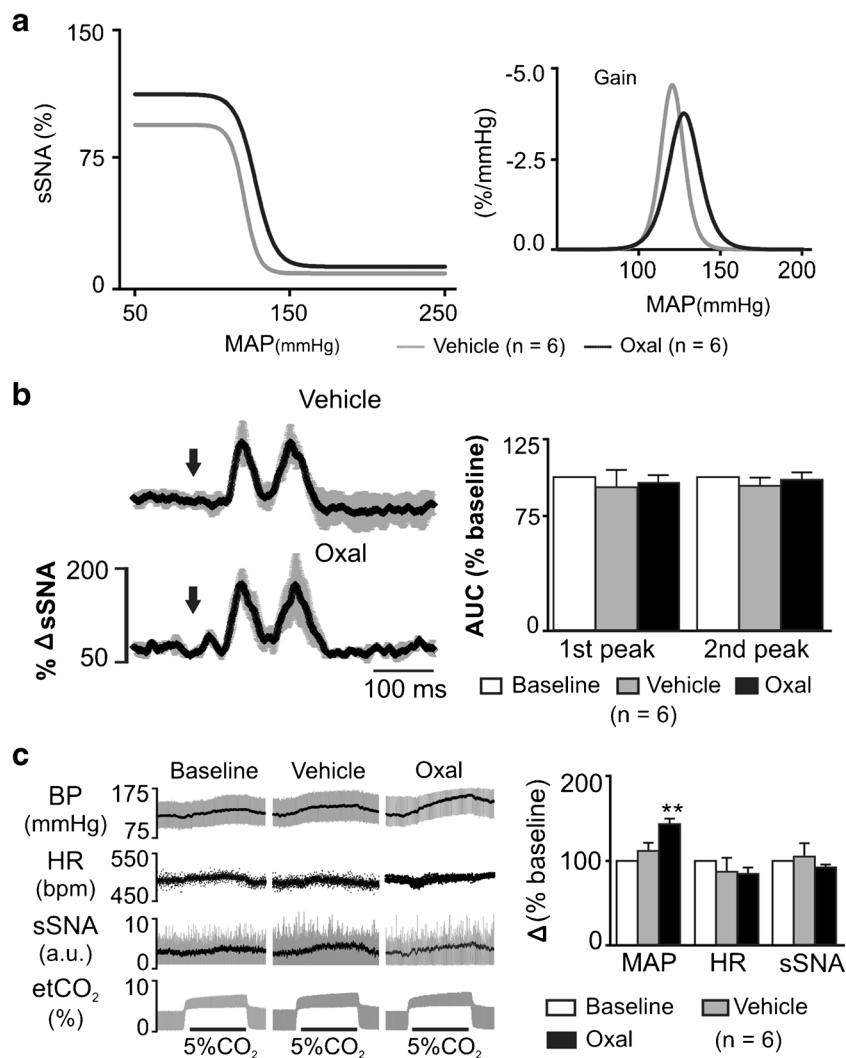

Fig. 4 Effect of acute oxaliplatin (Oxal) on the arterial baro-, somatosympathetic and central chemoreceptor reflexes. **a**. Average splanchnic sympathetic baroreflex function curves generated for data from intravenous injection of sodium nitroprusside (SNP) and phenylephrine hydrochloride (PE) following injection of the vehicle or oxaliplatin. Trace at right represents baroreflex gain for splanchnic sympathetic nerve activity (sSNA) (error bars are omitted for clarity—see Table 2). **b** Grouped effect of sciatic nerve-evoked stimulation of sSNA following vehicle and oxaliplatin injection. Data are mean (black) ± SE (grey). Arrows indicate the time of stimulation. Trace at right represents grouped data illustrating the changes in the 1st and 2nd sympathoexcitatory peaks following injections of the vehicle and acute oxaliplatin compared to control. **c** Experimental recording of hypercapnic episodes with 5% CO_2 in oxygen for 3 min at baseline (control) and following injections of the vehicle and acute oxaliplatin. Right trace represents grouped data of percentage changes in peak effects on cardiovascular (mean arterial pressure (MAP), heart rate (HR: bpm, beats per minute)) and sSNA parameters. Note that only pressor response to hypercapnia is significantly increased following acute Oxal injection compared to vehicle. Values are expressed as mean ± SE. **$P < 0.01$ compared to vehicle

In the vehicle-treated group, acute injection of oxaliplatin significantly increased the pressor response to central chemoreceptor activation by hypercapnia by 44% ($P < 0.01$) of the control, without any significant alteration in the HR and sympathoexcitatory responses ($n = 6$, Fig. 4c). Vehicle injection caused no significant alterations of MAP, HR or sSNA responses to hypercapnia ($n = 6$, Fig. 4c).

Effects of a single-dose oxaliplatin injection in the oxaliplatin-treated group

Effects of a single dose of oxaliplatin on cardiorespiratory parameters

In the treatment group ($n = 6$), injection of a single dose of oxaliplatin (i.v.) significantly increased MAP (16 ± 2.7 mmHg vs 2 ± 0.3 mmHg, $P < 0.01$) and sSNA ($28.3 \pm 4.0\%$ vs $2.1 \pm 0.6\%$, $P < 0.01$) compared to vehicle injection (Fig. 5). However, HR, PNf and PNamp were not significantly altered following single-dose oxaliplatin compared to vehicle in the treatment group ($n = 6$; Fig. 5).

Effects of a single dose of oxaliplatin on baroreceptor, somatosympathetic and central chemoreceptor reflexes

In the treatment group, a single-dose oxaliplatin injection (i.v.) significantly enhanced the upper plateau, range of sSNA and operating range (Table 3). Meanwhile, it reduced the lower plateau and the threshold level, without significantly altering midpoint, maximum gain, gain coefficient and the saturation level as compared to the vehicle ($n = 6$; Fig. 6a and Table 3).

The latencies of the first and second excitatory peaks of the somatosympathetic reflex were not significantly altered following injection of a single dose of oxaliplatin (80 ± 1 ms and 169 ± 4 ms, $n = 6$, ns) or vehicle (82 ± 3 ms and 173 ± 5 ms, $n = 6$, ns) as compared to baseline (78 ± 1 ms and 167 ± 5 ms). But the changes of heights of both excitatory peaks were significantly reduced by 33% ($P < 0.001$) and 32% ($P < 0.01$) of baseline, respectively, following a single dose of oxaliplatin injection as compared to baseline ($n = 6$, Fig. 6b). Injection of vehicle caused no alteration in peak heights of the somatosympathetic reflex (Fig. 6b).

The increased response in MAP, HR and sSNA to activation of central chemoreceptors by hypercapnia was not altered significantly following vehicle injection in the treatment group as compared to baseline. On the other hand, a single dose of oxaliplatin markedly potentiated the effects of hypercapnia on MAP by 56% ($P < 0.01$) and sSNA by 50% ($P < 0.01$) of baseline, without any significant alteration in the HR response ($n = 6$, Fig. 6c).

Table 2 Parameters describing baroreflex control of sSNA after acute i.v. injection of a single dose of vehicle and oxaliplatin in sham-treated rats

	Lower plateau (%)	Upper plateau (%)	Mid point (mmHg)	Max. gain (%/mmHg)	Gain coefficient	Range of sSNA (%)	Threshold level (mmHg)	Saturation level (mmHg)	Operating range (mmHg)
Vehicle	9.3 ± 1.4	99.9 ± 5.2	126.5 ± 4.3	−4.5 ± 1.5	0.19 ± 0.06	90.6 ± 3.9	116.4 ± 1.6	143.2 ± 9.0	30.2 ± 6.4
Oxaliplatin	11.1 ± 3.9 (ns)	111.9 ± 7.1 (P<0.05)	111.9 ± 5.5 (ns)	−3.8 ± 0.6 (ns)	0.14 ± 0.02 (ns)	103.6 ± 5.5 (ns)	105.5 ± 7.1 (ns)	137.3 ± 8.4 (ns)	38.5 ± 4.6 (P<0.05)

	Lower plateau (%)	Upper plateau (%)	Mid point (mmHg)	Max. gain (%/mmHg)	Range of sSNA (%)	Threshold level (mmHg)	Saturation level (mmHg)	Operating range (mmHg)
Vehicle	9.3 ± 1.4	99.9 ± 5.2	126.5 ± 4.3	−4.5 ± 1.5	90.6 ± 3.9	116.4 ± 1.6	143.2 ± 9.0	30.2 ± 6.4
Oxaliplatin	11.1 ± 3.9 (ns)	111.9 ± 7.1 (P<0.05)	111.9 ± 5.5 (ns)	−3.8 ± 0.6 (ns)	103.6 ± 5.5 (ns)	105.5 ± 7.1 (ns)	137.3 ± 8.4 (ns)	38.5 ± 4.6 (P<0.05)

Values are means ± SEM ($n = 6$). Maximum (Max.) gain is the slope of the sigmoid curve of best fit at the MAP corresponding to the steepest part of the curve

ns non-significant

Platinum accumulation within the rat brain following chronic oxaliplatin treatment

To determine whether platinum from oxaliplatin accumulates within the brain following treatment, the AAS technique was employed. A significant amount of platinum was detected in the brain from the oxaliplatin-treated animals (0.52 ppm ± 0.03, $P < 0.001$) when compared to the vehicle-treated cohort (all negative) ($n = 4$/group, Fig. 7).

Discussion

The novel findings of this study are that chronic oxaliplatin treatment has significant effects on cardiorespiratory function: (i) increases baseline MAP, sSNA and PNf but reduces HR and PNamp; (ii) delays the latencies of both excitatory peaks of the somatosympathetic reflex and attenuates heights of both peaks; (iii) reduces the baroreceptor sensitivity; (iv) increases the tachycardiac and sympathoexcitatory responses to hypercapnia; and (v) causes accumulation of platinum in the brain. A single dose of oxaliplatin (vi) increases MAP and sSNA; (vii) reduces both sympathoexcitatory peaks of the somatosympathetic reflex; and (viii) potentiates the pressor and sympathoexcitatory response to activation of the central chemoreflex. Another important finding of the study is a significant accumulation of platinum in the brain following chronic oxaliplatin treatment.

Hypertension is a common problem in patients treated with oxaliplatin chemotherapy [18]. Dermitzakis et al. [6] reported that oxaliplatin-based regimens, FOLFOX (oxaliplatin with 5-fluorouracil/leucovorin) or XELOX (oxaliplatin with capecitabine), significantly affect adrenergic cardiovascular reaction and parasympathetic heart innervation. However, these findings were obtained by measuring BP and cardiac rhythm in response to posture change. It has not been distinguished that the effects on ANS were solely due to oxaliplatin. Oxaliplatin also causes bradycardia, arrhythmias and changes in the electrocardiogram (ECG) as shown in canine experiments [9]. Cancer patients with metastases experience shortness of breath due to the spread of cancer to the lungs. These cardiorespiratory problems may be exacerbated by chemotherapy. However, the toxicity potential of oxaliplatin in the cardiovascular function and breathing has not been extensively investigated. Here, we report that oxaliplatin enhances BP, sSNA and the frequency of phrenic nerves. Phrenic nerves control the contraction of the diaphragm, and thereby allow proper functioning of the lungs and help us to breathe. From the effects of chronic oxaliplatin treatment in this study, we suggest that an increase in MAP is due to oxaliplatin-induced potentiation of sSNA, and the bradycardia is due to reflex response to increased sympathoexcitation or increase in the parasympathetic vagus nerve activity (Rahman, et al., unpublished data).

Peripheral neuropathy, the most common and dose-limiting complication of oxaliplatin, can affect sensory nerves and pain reflexes. Of the patients receiving oxaliplatin, 10–
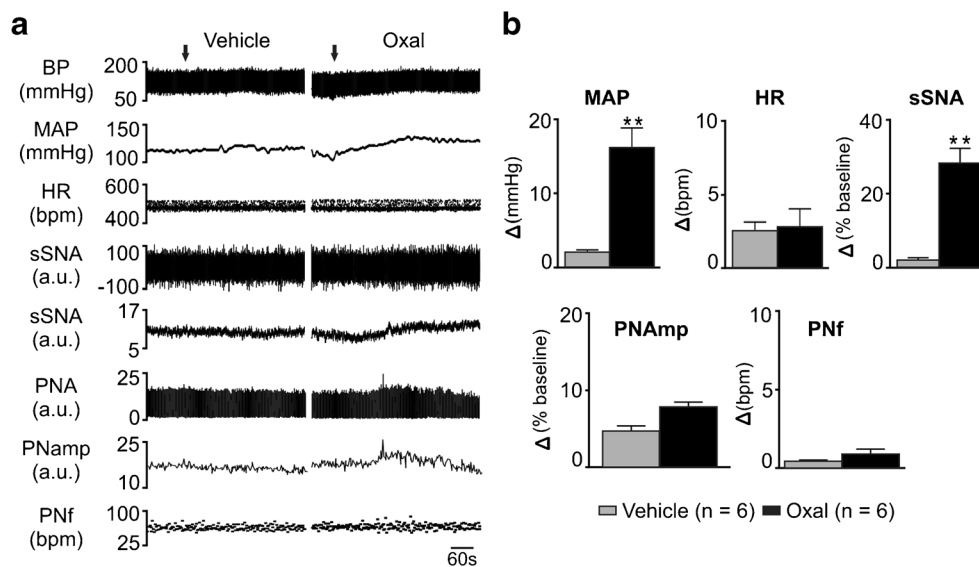

Fig. 5 Effect of a single dose oxaliplatin (Oxal) in chronic oxaliplatin-treated rats on cardiorespiratory parameters. a Representative recording of blood pressure (BP) (pulsatile and mean), heart rate (HR), splanchnic sympathetic nerve activity (sSNA) and phrenic nerve activity (PNA) (rectified) [arbitrary units (a.u.)], phrenic nerve frequency (PNf), and phrenic nerve amplitude (PNamp) before and after injection of vehicle or oxaliplatin. b Grouped data of maximum cardiovascular and respiratory effects following injections of vehicle and single dose of oxaliplatin. Peak effects are shown as absolute (mean arterial pressure (MAP), HR (bpm, beats per minute), PNf (bpm, bursts per minute)) or percentage (sSNA, PNamp) change from respective basal values. Single dose of Oxal causes a greater increase in MAP and sSNA compared to vehicle. Data are expressed as mean ± SE. The number of animals is shown in parentheses. **$P < 0.01$ compared with vehicle

Table 3 Parameters describing baroreflex control of sSNA after i.v. injection of a single dose of vehicle and oxaliplatin in oxaliplatin-treated rats

	Lower plateau (%)	Upper plateau (%)	Mid point (mmHg)	Max. gain (%/mmHg)	Gain coefficient	Range of sSNA (%)	Threshold level (mmHg)	Saturation level (mmHg)	Operating range (mmHg)
Vehicle	21.2±3.8	121.9±6.8	128.9±6.9	−3.6±0.7	0.14±0.04	103.4±7.9	90.5±9.5	149.1±9.4	56.9±9.4
Oxaliplatin	11.7±3.2 (P<0.01)	138.1±7.9 (P<0.05)	119.4±7.8 (ns)	−3.3±0.9 (ns)	0.12±0.05 (ns)	122.9±12.6 (P<0.05)	77.0±8.7 (P<0.05)	149.3±9.0 (ns)	80.8±9.2 (P<0.05)

	Lower plateau (%)	Upper plateau (%)	Mid point (mmHg)	Max. gain (%/mmHg)	Range of sSNA (%)	Threshold level (mmHg)	Saturation level (mmHg)	Operating range (mmHg)
Vehicle	21.2±3.8	121.9±6.8	128.9±6.9	−3.6±0.7	103.4±7.9	90.5±9.5	149.1±9.4	56.9±9.4
Oxaliplatin	11.7±3.2 (P<0.01)	138.1±7.9 (P<0.05)	119.4±7.8 (ns)	−3.3±0.9 (ns)	122.9±12.6 (P<0.05)	77.0±8.7 (P<0.05)	149.3±9.0 (ns)	80.8±9.2 (P<0.05)

Values are means ± SEM ($n = 6$). Maximum (Max.) gain is the slope of the sigmoid curve of best fit at the MAP corresponding to the steepest part of the curve

ns non-significant

15% suffer from sensory neurotoxicity as indicated by dysesthesia and paraesthesia [38]. These symptoms increase in intensity with cumulative oxaliplatin dose, leading to impaired sensation and/or deficit in fine sensory-motor coordination. However, the exact mechanism behind this neurotoxicity is still unclear. Following oxaliplatin treatment, platinum was detected in the DRG and sciatic nerve. Oxaliplatin also reduced sensory nerve conduction velocity in the sciatic nerve in rats [4]. Moreover, apoptosis in the DRG was observed in an in vitro preparation following oxaliplatin administration [45]. In accordance with these findings, our study revealed prolongation of the onset of sympathoexcitatory peaks and attenuation of both peak heights. These results may be attributed to oxaliplatin-induced damage of the sensory pathway.

The chemoreflex is an important mechanism altering both sympathetic function and thereby regulating sympathetic drive. Activation of the chemoreflex plays an important role in the development of emesis [46]. In our study, chronic oxaliplatin treatment augments the sympathoexcitatory response to chemoreceptor activation. This oxaliplatin-induced hyperactivation of the chemoreflex circuit may be correlated with the high incidence of vomiting associated with oxaliplatin chemotherapy. Augmented sympathetic excitatory chemoreceptor reflex also takes part in the potentiation of the sympathetic drive [28], as observed after chronic oxaliplatin injection in this study.

Oxidative stress may be a potential mechanism underlying platinum neurotoxicity [7], and recently, we reported that oxaliplatin increases oxidative stress in enteric neurons of mice [25]. Oxaliplatin also induced mitochondrial damage that leads to excessive nitric oxide (NO) production via activation of nitric oxide synthase [25]. NO acts as a neurotransmitter and is an important signalling molecule involved in the signalling pathways operating between cerebral blood vessels, neurons and glial cells that thereby participate in the regulation of cardiorespiratory functions [15]. NO production is increased in the response to increased CO_2 levels (hypercapnia) in human cell cultures [12] indicating its role in central chemoreflex. Similarly, oxaliplatin can induce NO alteration which in turn can affect the autonomic pathway to regulate cardiovascular function and breathing.

Baroreflex provides a rapid negative feedback loop to the BP change and maintains it at a constant level via the ANS. Recently, Dampney (2017) suggested that the baroreflex is reset in different circumstances including exercise, stress and sleep [5]. The same can be applicable to different disease states. Neurotoxicity, cardiotoxicity, hypertension and breathing dysfunction are common and severe adverse effects of cancer chemotherapy [6, 8, 18]. However, the effect of oxaliplatin chemotherapy on baroreflex function has not been studied before. Our novel results demonstrate a reduction in the maximum gain but an increase in the upper plateau and operating range in oxaliplatin-treated rats. It has been

suggested that to regulate BP at a level appropriate for a particular state or disease, four parameters of baroreflex function curve, including operating range, upper plateau, maximum gain and lower plateau, can be reset alone or in combination [5]. However, the exact way of baroreflex reset in a particular disease, and the mechanisms regulating the reset, are not completely understood. For example, the gain of the baroreflex curve is reduced in patients with sleep apneoa [27], but remains unchanged in essential hypertension [16, 22, 34]. In this study, although the baroreflex gain is reduced in oxaliplatin-treated rats, there is an evident increase in the upper plateau and operating range suggesting that baroreflex can function over a higher range of BP. Moreover, midpoint MAP in oxaliplatin-treated rats shows a trend to a higher value compared to sham-treated rats (even though the
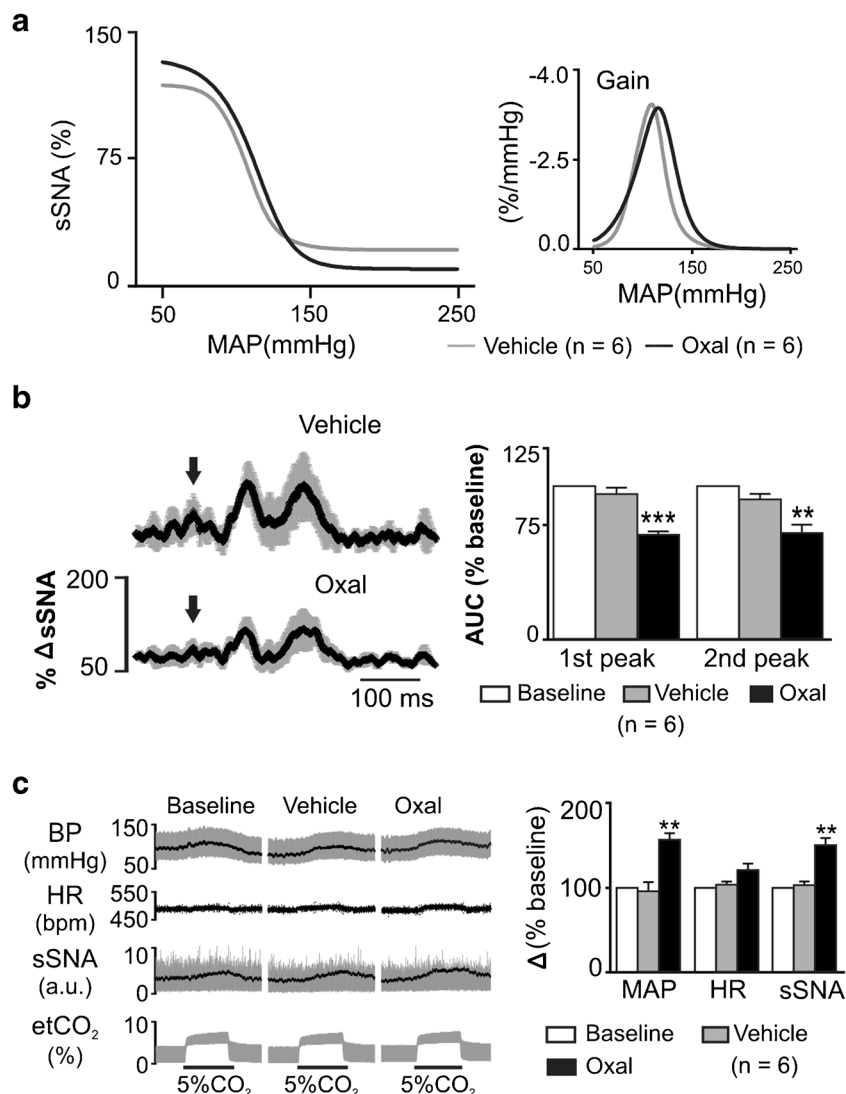

Fig. 6 Effect of a single-dose oxaliplatin (Oxal) in chronic oxaliplatin-treated rats on the arterial baro-, somatosympathetic and central chemoreceptor reflexes. **a** Average splanchnic sympathetic baroreflex function curves generated for data from intravenous injection of sodium nitroprusside (SNP) and phenylephrine hydrochloride (PE) following injection of vehicle or oxaliplatin. Trace at right represents baroreflex gain for splanchnic sympathetic nerve activity (sSNA) (error bars are omitted for clarity—see Table 3). **b** Grouped effect of sciatic nerve-evoked stimulation of sSNA following vehicle and oxaliplatin injection. Data are mean (black) ± SE (grey). Arrows indicate the time of stimulation. Trace at right represents grouped data illustrating the changes in the 1st and 2nd sympathoexcitatory peaks following injections of vehicle and acute oxaliplatin compared to control. Following injection of a single-dose Oxal, both the sympathoexcitatory peaks of the somatosympathetic reflex are reduced compared to vehicle. **c** Experimental recording of hypercapnic episodes with 5% CO_2 in oxygen for 3 min at baseline (control) and following injections of vehicle and acute oxaliplatin. Right trace represents grouped data of percentage changes in peak effects on cardiovascular parameters (mean arterial pressure (MAP), heart rate (HR: bpm, beats per minute) and sSNA (a.u., arbitrary units)) in response to hypercapnia after injection of the vehicle or oxaliplatin. The pressor and sympathoexcitatory responses to hypercapnia are potentially increased following a single-dose Oxal injection compared to vehicle injection. Values are expressed as mean ± SE. ***$P < 0.001$, **$P < 0.01$ compared to control
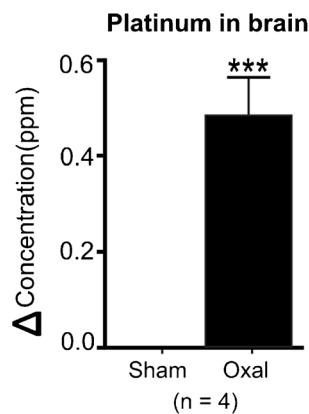
Platinum in brain

Fig. 7 Platinum accumulation within the brain following chronic oxaliplatin treatment. Grouped data representing platinum concentration in the rat brain in sham and oxaliplatin-treated rats. A significant amount of platinum (ppm, parts per million) was detected within the rat brain following oxaliplatin treatment, when compared to the vehicle treatment. Data are expressed as mean ± SE. Number of animals are shown in parentheses. *$P < 0.001$ compared to vehicle-treated group

difference is not significant). Further detailed studies can help in understanding the mechanisms behind this.

Most of the adverse effects of chemotherapy are cumulative and occur after chronic use of the drug. Along with cumulative, chronic side effects, oxaliplatin also causes transient acute neuropathy, to sensory nerves in particular [6]. Little is known about the acute effects of oxaliplatin on nerve excitability. Adelsberger et al. [3] reported an alteration in the activation and inactivation behaviour of Na^+ channels pointing towards a direct effect on nerve excitability. However, the exact mechanism is still unclear. We have observed a significant change in cardiorespiratory parameters, on both sympathetic and phrenic nerve functions and sympathetic reflexes after chronic treatment with oxaliplatin over 14 days. But acute i.v. injection of oxaliplatin causes no alteration in any of the cardiorespiratory parameters or cardiovascular reflexes. However, i.v. injection of a single dose of oxaliplatin in chronic oxaliplatin-treated rats alters cardiovascular and respiratory functions along with the sympathetic reflexes. One possible explanation is that the cumulative dose administered over a long period of time may allow for the accumulation of platinum metabolites that then enter cells via a combination of passive and facilitated diffusion. And further i.v. injection of a single dose of oxaliplatin possibly causes alteration in the sensitivity of the autonomic nerves and a different reflex arch produced by cumulative, chronic oxaliplatin treatment. This explanation is supported by previous studies where platinum has been detected in the brain or periphery several days or weeks after treatment with oxaliplatin with a minimum gap of 3 days [10, 17, 39, 40].

There are few studies determining the amount of platinum accumulation in the brain, spinal cord, DRG and peripheral nerves. Those studies reported higher platinum accumulation in DRG and peripheral nerves while a very low amount

accumulated in the brain and spinal cord after chronic treatment with cisplatin and oxaliplatin [17, 39, 40]. Consistent with previous reports, our study also reveals that chronic oxaliplatin treatment results in platinum accumulation in the brain. This platinum accumulation can be correlated with the effects of oxaliplatin on the ANS regulating cardiorespiratory functions and sympathetic reflexes as observed in this study. However, it is not clear whether the small amount of platinum is sufficient to cause any alteration in the nervous system. The possible explanation is that a different amount of platinum is required to induce a certain grade of neurotoxicity in different sites of accumulation. For example, 2 μg g^{-1} platinum was required to show mild toxicity in dorsal roots, whereas 0.5 μg g^{-1} was enough to show the same degree of toxicity in the DRG after cisplatin treatment [17]. The amount of platinum found (0.5 μg g^{-1}) in this study is higher than that found (less than 100 ng g^{-1}) in a previous study [39] in spite of the shorter duration of oxaliplatin treatment (2 weeks) in this study. The previous study used 1 mg kg^{-1} oxaliplatin for 8 weeks. As part of the brainstem (area postrema) lacks a true blood-brain barrier, this may act as a conduit for greater platinum drug entry to this particular brain region. As the brainstem contains the cardiorespiratory control centres, this may explain why autonomic functions are particularly susceptible to change following oxaliplatin treatment. The histopathological effects of oxaliplatin treatment on these cardiorespiratory centres are part of an ongoing study. Moreover, chemotherapy treatment that lasts for at least 1 year may result in the accumulation of more amount of platinum in the brain leading to greater toxicity. It has been shown that the degree of neurotoxicity of platinum drugs is not correlated with their solubility or concentration of platinum [40]. Therefore, it is suggested that mechanisms other than the tissue accumulation of platinum, e.g. relative reactivity of platinum compounds with plasma proteins, may be involved in the neurotoxicity of platinum drugs.

Conclusion

Regardless of therapeutic advances, cancer survivors remain at higher risk of cardiovascular morbidity and mortality due to the disease itself or related treatment strategy [26, 41]. Oxaliplatin treatment induces severe peripheral neurotoxicity and cardiotoxicity as well as gastrointestinal side effects including nausea and vomiting that often demand dose limitations/withdrawal of the treatment. The mechanisms underlying these toxicities are not clearly understood. Oxaliplatin-induced platinum accumulation may be associated with its toxicity to cardiovascular, respiratory and other neuronal functions. Our findings are that oxaliplatin, after cumulative, chronic administration, increases basal MAP, sSNA and PNf. Oxaliplatin also reduces baroreceptor sensitivity and somatosympathetic reflex suggesting a role of oxaliplatin in

autonomic function. Further studies elucidating mechanisms of chronic oxaliplatin-induced autonomic dysfunction causing an increase in MAP, sympathoexcitation and reduced barosensitivity are warranted in order to develop strategies to reduce the risk of cardiovascular mortality and achieve more effective anti-cancer treatment. At the least, the present data suggest that there may be a need to avoid chronic dosing with platinum compounds.

Compliance with ethical standards

Conflict of interest　The authors declare that they have no conflicts of interest.

References

1. Abbott SB, Pilowsky PM (2009) Galanin microinjection into rostral ventrolateral medulla of the rat is hypotensive and attenuates sympathetic chemoreflex. Am J Physiol Regul Integr Comp Physiol 296:R1019–R1026. https://doi.org/10.1152/ajpregu.90885.2008

2. Adams SC, Schondorf R, Benoit J, Kilgour RD (2015) Impact of cancer and chemotherapy on autonomic nervous system function and cardiovascular reactivity in young adults with cancer: a case-controlled feasibility study. BMC Cancer 15:414. https://doi.org/10.1186/s12885-015-1418-3

3. Adelsberger H, Quasthoff S, Grosskreutz J, Lepier A, Eckel F, Lersch C (2000) The chemotherapeutic oxaliplatin alters voltage-gated Na(+) channel kinetics on rat sensory neurons. Eur J Pharmacol 406:25–32

4. Cavaletti G, Tredici G, Petruccioli MG, Donde E, Tredici P, Marmiroli P, Minoia C, Ronchi A, Bayssas M, Etienne GG (2001) Effects of different schedules of oxaliplatin treatment on the peripheral nervous system of the rat. Eur J Cancer 37:2457–2463

5. Dampney RAL (2017) Resetting of the baroreflex control of sympathetic vasomotor activity during natural behaviors: description and conceptual model of central mechanisms. Front Neurosci 11. https://doi.org/10.3389/fnins.2017.00461

6. Dermitzakis EV, Kimiskidis VK, Eleftheraki A, Lazaridis G, Konstantis A, Basdanis G, Tsiptsios I, Georgiadis G, Fountzilas G (2014) The impact of oxaliplatin-based chemotherapy for colorectal cancer on the autonomous nervous system. Eur J Neurol 21:1471–1477. https://doi.org/10.1111/ene.12514

7. Di Cesare ML, Zanardelli M, Failli P, Ghelardini C (2012) Oxaliplatin-induced neuropathy: oxidative stress as pathological mechanism. Protective effect of silibinin. J Pain 13:276–284. https://doi.org/10.1016/j.jpain.2011.11.009

8. El-Awady ESE, Moustafa YM, Abo-Elmatty DM, Radwan A (2011) Cisplatin-induced cardiotoxicity: mechanisms and

9. cardioprotective strategies. Eur J Pharmacol 650:335–341. https://doi.org/10.1016/j.ejphar.2010.09.085

9. Eloxatin (2014) Product monograph. http://products.sanofi.ca/en/eloxatin.pdf. (accessed 2/2014)

10. Esteban-Fernandez D, Verdaguer JM, Ramirez-Camacho R, Palacios MA, Gomez-Gomez MM (2008) Accumulation, fractionation, and analysis of platinum in toxicologically affected tissues after cisplatin, oxaliplatin, and carboplatin administration. J Anal Toxicol 32:140–146

11. Extra JM, Marty M, Brienza S, Misset JL (1998) Pharmacokinetics and safety profile of oxaliplatin. Semin Oncol 25:13–22

12. Fathi AR, Yang C, Bakhtian KD, Qi M, Lonser RR, Pluta RM (2011) Carbon dioxide influence on nitric oxide production in endothelial cells and astrocytes: cellular mechanisms. Brain Res 1386:50–57. https://doi.org/10.1016/j.brainres.2011.02.066

13. Floyd JD, Nguyen DT, Lobins RL, Bashir Q, Doll DC, Perry MC (2005) Cardiotoxicity of cancer therapy. J Clin Oncol 23:7685–7696. https://doi.org/10.1200/jco.2005.08.789

14. Gamelin E, Gamelin L, Bossi L, Quasthoff S (2002) Clinical aspects and molecular basis of oxaliplatin neurotoxicity: current management and development of preventive measures. Semin Oncol 29:21–33. https://doi.org/10.1053/sonc.2002.35525

15. Garry PS, Ezra M, Rowland MJ, Westbrook J, Pattinson KT (2015) The role of the nitric oxide pathway in brain injury and its treatment–from bench to bedside. Exp Neurol 263:235–243. https://doi.org/10.1016/j.expneurol.2014.10.017

16. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G (1998) Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 31:68–72. https://doi.org/10.1161/01.hyp.31.1.68

17. Gregg RW, Molepo JM, Monpetit VJ, Mikael NZ, Redmond D, Gadia M, Stewart DJ (1992) Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. J Clin Oncol 10:795–803. https://doi.org/10.1200/jco.1992.10.5.795

18. Han C, McKeage M (2012) Neuropathies associated with oxaliplatin therapy. Asia Pac J Clin Oncol 8:107–110. https://doi.org/10.1111/j.1743-7563.2012.01547.x

19. Hind D, Tappenden P, Tumur I, Eggington S, Sutcliffe P, Ryan A (2008) The use of irinotecan, oxaliplatin and raltitrexed for the treatment of advanced colorectal cancer: systematic review and economic evaluation. Health Technol Assess 12:iii-ix–xi-162

20. Kent BB, Drane JW, Blumenstein B, Manning JW (1972) A mathematical model to assess changes in the baroreceptor reflex. Cardiology 57:295–310

21. Ketch T, Biaggioni I, Robertson R, Robertson D (2002) Four faces of baroreflex failure: hypertensive crisis, volatile hypertension, orthostatic tachycardia, and malignant vagotonia. Circulation 105:2518–2523

22. Mancia G, Ludbrook J, Ferrari A, Gregorini L, Zanchetti A (1978) Baroreceptor reflexes in human hypertension. Circ Res 43:170–177. https://doi.org/10.1161/01.RES.43.2.170

23. 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. https://doi.org/10.1016/j.nbd.2004.09.013

24. McDowall LM, Dampney RA (2006) Calculation of threshold and saturation points of sigmoidal baroreflex function curves. Am J Phys Heart Circ Phys 291:H2003–H2007. https://doi.org/10.1152/ajpheart.00219.2006

25. McQuade RM, Carbone SE, Stojanovska V, Rahman A, Gwynne RM, Robinson AM, Goodman CA, Bornstein JC, Nurgali K (2016) Role of oxidative stress in oxaliplatin-induced enteric neuropathy and colonic dysmotility in mice. Br J Pharmacol 173:3502–3521. https://doi.org/10.1111/bph.13646

26. Monsuez JJ, Charniot JC, Vignat N, Artigou JY (2010) Cardiac side-effects of cancer chemotherapy. Int J Cardiol 144:3–15. https://doi.org/10.1016/j.ijcard.2010.03.003

27. Narkiewicz K, Pesek CA, Kato M, Phillips BG, Davison DE, Somers VK (1998) Baroreflex control of sympathetic nerve activity and heart rate in obstructive sleep apnea. Hypertension 32:1039–1043. https://doi.org/10.1161/01.hyp.32.6.1039

28. Narkiewicz K, Pesek CA, van de Borne PJ, Kato M, Somers VK (1999) Enhanced sympathetic and ventilatory responses to central chemoreflex activation in heart failure. Circulation 100:262–267

29. Nedoboy PE, Mohammed S, Kapoor K, Bhandare AM, Farnham MM, Pilowsky PM (2016) pSer40 tyrosine hydroxylase immuno-histochemistry identifies the anatomical location of C1 neurons in rat RVLM that are activated by hypotension. Neuroscience 317: 162–172. https://doi.org/10.1016/j.neuroscience.2016.01.012

30. Rahman AA, Shahid IZ, Pilowsky PM (2011) Intrathecal neuromedin U induces biphasic effects on sympathetic vasomotor tone, increases respiratory drive and attenuates sympathetic reflexes in rat. Br J Pharmacol 164:617–631

31. Rahman AA, Shahid IZ, Pilowsky PM (2012) Differential cardio-respiratory and sympathetic reflex responses to microinjection of neuromedin U in rat rostral ventrolateral medulla. J Pharmacol Exp Ther 341:213–224

32. Rahman AA, Shahid IZ, Pilowsky PM (2013) Neuromedin U causes biphasic cardiovascular effects and impairs baroreflex function in rostral ventrolateral medulla of spontaneously hypertensive rat. Peptides 44:15–24. https://doi.org/10.1016/j.peptides.2013.03.017

33. Raymond E, Faivre S, Woynarowski JM, Chaney SG (1998) Oxaliplatin: mechanism of action and antineoplastic activity. Semin Oncol 25:4–12

34. Rea RF, Hamdan M (1990) Baroreflex control of muscle sympathetic nerve activity in borderline hypertension. Circulation 82: 856–862. https://doi.org/10.1161/01.CIR.82.3.856

35. Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. FASEB J 22:659–661. https://doi.org/10.1096/fj.07-9574LSF

36. Renn CL, Carozzi VA, Rhee P, Gallop D, Dorsey SG, Cavaletti G (2011) Multimodal assessment of painful peripheral neuropathy induced by chronic oxaliplatin-based chemotherapy in mice. Mol Pain 7:29. https://doi.org/10.1186/1744-8069-7-29

37. Rothenberg ML, Oza AM, Bigelow RH, Berlin JD, Marshall JL, Ramanathan RK, Hart LL, Gupta S, Garay CA, Burger BG, Le Bail N, Haller DG (2003) Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol 21: 2059–2069. https://doi.org/10.1200/jco.2003.11.126

38. Saif MW, Reardon J (2005) Management of oxaliplatin-induced peripheral neuropathy. Ther Clin Risk Manag 1:249–258

39. Screnci D, Er HM, Hambley TW, Galettis P, Brouwer W, McKeage MJ (1997) Stereoselective peripheral sensory neurotoxicity of diaminocyclohexane platinum enantiomers related to ormaplatin and oxaliplatin. Br J Cancer 76:502–510

40. Screnci D, McKeage MJ, Galettis P, Hambley TW, Palmer BD, Baguley BC (2000) Relationships between hydrophobicity, reactivity, accumulation and peripheral nerve toxicity of a series of platinum drugs. Br J Cancer 82:966–972. https://doi.org/10.1054/bjoc.1999.1026

41. Senkus E, Jassem J (2011) Cardiovascular effects of systemic cancer treatment. Cancer Treat Rev 37:300–311. https://doi.org/10.1016/j.ctrv.2010.11.001

42. Shahid IZ, Rahman AA, Pilowsky PM (2012) Orexin A in rat rostral ventrolateral medulla is pressor, sympatho-excitatory, increases barosensitivity and attenuates the somato-sympathetic reflex. Br J Pharmacol 165:2292–2303

43. Slade JH, Alattar ML, Fogelman DR, Overman MJ, Agarwal A, Maru DM, Coulson RL, Charnsangavej C, Vauthey JN, Wolff RA, Kopetz S (2009) Portal hypertension associated with oxaliplatin administration: clinical manifestations of hepatic sinusoidal injury. Clin Colorectal Cancer 8:225–230. https://doi.org/10.3816/CCC.2009.n.038

44. Sorensen JC, Petersen AC, Timpani CA, Campelj DG, Cook J, Trewin AJ, Stojanovska V, Stewart M, Hayes A, Rybalka E (2017) BGP-15 protects against oxaliplatin-induced skeletal myopathy and mitochondrial reactive oxygen species production in mice. Front Pharmacol 8:137. https://doi.org/10.3389/fphar.2017.00137

45. Ta LE, Espeset L, Podratz J, Windebank AJ (2006) Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology 27:992–1002. https://doi.org/10.1016/j.neuro.2006.04.010

46. Uchino M, Kuwahara M, Ebukuro S, Tsubone H (2006) Modulation of emetic response by carotid baro- and chemoreceptor activations. Auton Neurosci 128:25–36. https://doi.org/10.1016/j.autneu.2005.12.006

47. Yeh ET, Tong AT, Lenihan DJ, Yusuf SW, Swafford J, Champion C, Durand JB, Gibbs H, Zafarmand AA, Ewer MS (2004) Cardiovascular complications of cancer therapy: diagnosis, pathogenesis, and management. Circulation 109:3122–3131. https://doi.org/10.1161/01.cir.0000133187.74800.b9