Use of sodium bicarbonate to promote weight gain, maintain body temperature, normalize renal functions and prevent mortality in rodents receiving the chemotherapeutic agent cisplatin

Josée Guindon[1,2] and **Andrea G. Hohmann**[1,2]

[1]Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

[2]Department of Psychology, University of Georgia, Athens, GA, USA

SUMMARY

A simple method to reduce adverse effects of the chemotherapeutic agent cisplatin on animal health is described. Animals receiving normal saline (0.9% NaCl) s.c. prior to once weekly injections of cisplatin (3 mg/kg i.p.×3 or 4 weeks) exhibited failure of weight gain, lowered body temperature, elevations in creatinine and ketone levels and increased kidney weight ratios. By contrast, rats treated with sodium bicarbonate (4% $NaHCO_3$ in saline s.c.) prior to cisplatin (3 mg/kg i.p.×3 or 4 weeks) exhibited normal weight gain, body temperature, creatinine and ketone levels, as well as normal kidney weight ratios (over 16 or 28 days, respectively). Cisplatin-induced neuropathy (i.e. mechanical and cold allodynia) developed equivalently in both groups. Our studies suggest that $NaHCO_3$ pretreatment promotes animal health and prevents weight loss, body temperature dysregulation and signs of renal toxicity (i.e. increases in creatinine and kidney weight ratio) following repeated cisplatin treatment without altering the development of chemotherapy-induced peripheral neuropathy.

Cisplatin, a platinum-derived chemotherapeutic agent, produces both painful peripheral neuropathy and renal toxicity [1,20]. In rodent subjects, cisplatin is specifically used to model peripheral sensory neuropathies that develop in humans treated with this agent [4,5,8,20]. In animal models, cisplatin-induced mortality, attributable to damage to renal functions [22,30], ranges from 10–50% [6,27]. Attempts to minimize mortality in rodents involve reductions in frequency, duration and/or dosing of cisplatin [4 for a review].

[*]Corresponding authors at: Department of Psychological and Brain Sciences, Indiana University, 702 N Walnut Grove Ave, Bloomington, IN 47405-2204, jguindon@indiana.edu (J. Guindon), or hohmanna@indiana.edu (A.G. Hohmann). Dedicated to Manon Marcotte (mother of JG) who passed away January 3rd 2011 of lymphoma.

Conflict of interest
The authors state no conflict of interest.

Nonetheless, cisplatin produces changes in body temperature [6,27] and detrimental effects on body weight in rodent subjects [5,6,26,27].

Human studies have reported beneficial effects of sodium bicarbonate in reducing blood acidosis and kidney toxicity in chemotherapy patients [7,21]. Therefore, we developed a new preclinical method to minimize damage to renal functions (assessed by measurements of creatinine levels, kidney weight ratio and urine pH) and improve general health (weight gain, normal body temperature and reduced mortality) in rodent subjects. We evaluated whether $NaHCO_3$ (4% in 0.9% NaCl (saline)), administered subcutaneously (s.c.) immediately prior to cisplatin treatment, would prevent adverse side-effects (e.g. weight loss, lowered body temperature, creatinine increases, kidney weight ratio increases and mortality) associated with repeated cisplatin dosing. We hypothesized that concurrent administration of sodium bicarbonate (4 % $NaHCO_3$; pH 8.06 ± 0.01), an alkaline solution, would counteract acidic effects of cisplatin that underly nephrotoxicity and mortality in rodents, thereby producing a beneficial impact on animal health.

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA), weighing 254–382 g before testing, were used. Animals were single housed in standard plastic cages with sawdust bedding in a climate-controlled room (23°C and 45% humidity), and maintained under a 12h light (7am to 7pm)/dark cycle. Rats were given free access to standard rodent chow and water. Total of 242 rats were used. Experimental protocols were approved by the Institutional Animal Care and Use Committee and followed guidelines for the treatment of animals of the International Association for the Study of Pain [31].

Cisplatin (Tocris, Ellisville, MO, USA) was administered intraperitoneally (i.p.) once a week at a dose of 3 mg/kg for 3 (cumulative dose: 9 mg/kg i.p. over 16 days) or 4 (12 mg/kg i.p. over 28 days) weeks [6,11]. Cisplatin was diluted in normal saline (0.9 % NaCl). Saline [4] or 4% sodium bicarbonate ($NaHCO_3$ dissolved in saline) was administered (2 ml s.c.) before each i.p injection of cisplatin or saline. Injections were always performed after completion of mechanical and cold withdrawal testing.

Mechanical withdrawal thresholds were assessed using a digital Electrovonfrey Anesthesiometer (IITC Life Sciences, Woodland Hills, CA) equipped with a rigid tip [11]. Cold allodynia was assessed by applying drops of room temperature acetone to the plantar surface of the hind paw as previously described [11]. Mechanical withdrawal thresholds and cold withdrawal frequencies were measured every 4 days over 16 (for saline/cisplatin group) or 28 (for $NaHCO_3$/cisplatin group) days. Testing took place on days 0, 4, 8, 12, 16 in all groups and continued on days 20, 24, 28 in relevant cohorts.

Rectal temperature was assessed in animals receiving $NaHCO_3$ or saline pretreatments using a rectal probe (Physitemp RET-2 rectal probe for rats, Clifton, NJ, USA) and meter (Physitemp Model BAT-12R, Clifton, NJ, USA). Body temperature was recorded every four days. The same animals were used to evaluate mechanical and cold allodynia as well as body weight and core temperature changes. A subset (n = 8–9 per group) of these animals were used to evaluate kidney functions.

Creatinine, ketone and glucose levels (mg/dL) were measured in whole blood using the PTS CardioChek diagnostic apparatus (Cliawaived.com, San Diego, CA, USA). Urine and blood were extracted post mortem from the bladder and renal artery, respectively, using a 25 gauge needle and 1 ml syringe. Urine and blood pH was measured using a digital pH 110 meter (Oakton Instruments, Vermon Hills, IL, USA). Kidney weight ratio was also measured [9]. The experimenter was blinded to the experimental condition.

Paw withdrawal thresholds (mechanical) and frequencies (cold) were calculated for each paw and averaged. Data were analyzed using analysis of variance (ANOVA) for repeated measures or one-way ANOVA as appropriate. The Greenhouse-Geisser correction was applied to all repeated factors. The source of significant interactions was further evaluated by performing one way ANOVAs at each time point, followed by Bonferroni post hoc tests. Analyses were performed using SPSS statistical software (version 19.0; SPSS Incorporated, Chicago, IL, USA). $P < 0.05$ was considered significant.

No differences were observed between groups receiving saline/saline and $NaHCO_3$/saline treatments in body weight ($P > 0.526$), body temperature ($P > 0.942$), mechanical threshold ($P > 0.08$) or cold withdrawal frequency ($P > 0.620$) in either injection paradigm. Similarly, no differences in creatinine ($P > 0.6310$), ketone ($P > 0.5891$), glucose ($P > 0.2620$), urine pH ($P > 0.2819$), blood pH ($P > 0.3249$) or kidney weight ratios ($P > 0.0675$) were observed in groups receiving saline/saline or $NaHCO_3$/saline treatments. Therefore, these groups were pooled into a single control group (the control/saline group) for each survival time for further statistical analyses.

Weight gain was absent in animals receiving saline (in lieu of $NaHCO_3$) prior to cisplatin. By contrast, both control/saline and $NaHCO_3$/cisplatin-treated groups exhibited time-dependent increases in body weight in both injection paradigms ($F_{8,180} = 11.31$ $P < 0.0001$ (3 cisplatin cycles over 16 days; Fig. 1A) and ($F_{14,567} = 5.60$ $P < 0.0001$ (4 cisplatin cycles over 28 days; Fig. 1B). Body weight was lower in saline/cisplatin-treated groups ($F_{2,45} = 11.55$, $P < 0.0001$, Fig. 1A; $F_{2,81} = 3.17$, $P < 0.047$, Fig. 1B) relative to either control/saline or $NaHCO_3$/cisplatin groups. The magnitude and rate of weight gain did not differ in these latter groups. Weight gain appeared on day 8 and persisted throughout the 16 day observation interval ($P < 0.001$) (Fig. 1A) in the $NaHCO_3$/cisplatin group receiving 3 cycles of cisplatin. Weight gain appeared on day 12 and was maintained throughout the 28 day observation interval ($P < 0.045$) (Fig. 1B) in the $NaHCO_3$/cisplatin group receiving 4 cycles of cisplatin. Saline/cisplatin-treated groups also exhibited lower body temperature relative to either control/saline or $NaHCO_3$/cisplatin groups; lowered body temperature was observed on day 4 and was maintained throughout the study ($F_{2,45} = 15.35$, $P < 0.0001$; day 4–16 ($P < 0.011$); Fig. 1C) and ($F_{2,81} = 12.21$, $P < 0.0001$; day 4–24 ($P < 0.037$); Fig. 1D). Body temperature in $NaHCO_3$/cisplatin groups did not differ from that observed in control/saline groups ($P = 1.000$) at any observation interval. Thus, sodium bicarbonate treatment was protective against hypothermic effects of cisplatin.

Both saline/cisplatin- and $NaHCO_3$/cisplatin-treated groups developed equivalent levels of mechanical allodynia ($F_{2,45} = 1686.04$, $P < 0.0001$ (Fig. 2A) and ($F_{2,81} = 3805.20$, $P < 0.0001$ (Fig. 2B). Reductions in mechanical thresholds were observed in each cisplatin dosing paradigm relative to the control/saline group. Cisplatin-induced mechanical allodynia was present at all observation intervals ($P < 0.0001$; (Fig. 2A,B). Furthermore, both saline/cisplatin and $NaHCO_3$/cisplatin treatments increased paw withdrawal frequencies to acetone ($F_{2,45} = 372.87$, $P < 0.0001$ (Fig. 2C) and ($F_{2,81} = 1145.76$, $P < 0.0001$ (Fig. 2D), consistent with development of cold allodynia. Cisplatin-induced cold allodynia was present ($P < 0.0001$) at all observation intervals relative to the control/saline group (Fig. 2C,D).

Mortality was not observed in animals treated with the $NaHCO_3$/cisplatin dosing paradigm (n = 24 for 16 days; n = 44 for 28 days). By contrast, 11% (1 out of 9 rats in the 16 day dosing paradigm) and 20% (2 out of 10 rats in the 28 day dosing) died in the group receiving saline/cisplatin treatment (n = 8 for 16 days; n = 8 for 28 days), demonstrating toxicity that precluded further cisplatin dosing.

Saline/cisplatin treatment increased creatinine ($F_{2,22} = 21.97$, $P < 0.0001$, 16 days; $F_{2,21} = 14.48$, $P < 0.0001$, 28 days) (Fig. 3A,B) and ketone ($F_{2,22} = 18.48$, $P < 0.0001$, 16 days; $F_{2,21} = 15.51$, $P < 0.0001$, 28 days) (Fig. 3C,D) levels in whole blood relative to control/ saline treatment. These increases were prevented by $NaHCO_3$ pretreatment in the cisplatin-treated groups. Creatinine levels were similar in control/saline and $NaHCO_3$/cisplatin-treated groups ($P = 0.810$, at 16 and $P = 1.000$, at 28 days). By contrast, blood glucose levels ($F_{2,22} = 0.018$, $P = 0.982$, 16 days; $F_{2,21} = 0.02$, $P = 0.98$, 28 days) (Fig. 3E,F) did not differ in any group.

Cisplatin lowered urinary pH in saline/cisplatin-treated groups relative to control/saline groups in rats receiving 4 cycles of cisplatin and killed on day 28 post initial cisplatin dosing ($P < 0.001$) (Table 1). Urinary pH was not reduced in saline/cisplatin groups receiving 3 cycles of cisplatin and killed at day 16 following initial cisplatin dosing ($P = 0.402$) (Table 1). In cisplatin-treated animals, $NaHCO_3$ pretreatment increased the urinary pH ($F_{2,22} = 29.06$, $P < 0.0001$, 16 days; $F_{2,21} = 34.77$, $P < 0.0001$, 28 days) (Table 1) compared to either control/saline or saline/cisplatin groups in each dosing paradigm (Table 1). $NaHCO_3$ pretreatment also blocked the cisplatin-induced increase in kidney weight ratio (Table 1). The saline/cisplatin group had a higher kidney weight ratio at both 16 ($F_{2,22} = 18.92$, $P < 0.0001$) and 28 ($F_{2,21} = 8.38$, $P < 0.002$) day survival times relative to control/saline or $NaHCO_3$/cisplatin-treated groups. By contrast, the kidney weight ratio of $NaHCO_3$/ cisplatin-treated rats was similar to that observed in control/saline groups ($P = 1.000$, at 16 or 28 days). Thus, sodium bicarbonate prevented cisplatin-induced increases in kidney weight ratio. Blood pH ($F_{2,22} = 0.291$, $P = 0.750$, 16 days; $F_{2,21} = 2.71$, $P = 0.090$, 28 days) (Fig. 3E,F) did not differ in any of the groups.

Cisplatin causes dose-dependent renal toxicity, a major dose-limiting consequence of cisplatin treatment [22,30]. Nephrotoxicity is complex and becomes more severe with repeated dosing [6,22,27]. Cisplatin produces disturbances in water permeability [16] and infiltration of inflammatory cells [18,24]. Cisplatin is partially metabolized into toxic species [30], promoting an acidic environment that produces nephrotoxicity and changes in renal functions, documented by increases in creatinine (normal creatinine between 0.2 –1.36 mg/dL) and kidney weight ratio [13,15] in our study. A change in the body pH (normal blood pH: 7.35–7.45; normal urine pH ~7.00) toward acidity (below pH 7.0) favours oxygen to be driven out of the body, thereby facilitating cancer cell survival [10,12,21]. Thus, urine, but not blood, pH would be expected to be increased by alkaline and decreased by acidic diets/treatments, as observed in our study and elsewhere [21]. Cancerous tissue is acidic because cells spill lactic acid, causing an acidic pH [29]. Thus, using systemic buffers to manipulate acidification is beneficial as a cancer treatment strategy in humans [7,19,21,25]. Our preclinical findings with $NaHCO_3$ delivered immediately prior to cisplatin administration, confirm the clinical predictions. In our study, $NaHCO_3$ treatment was associated with normal renal functions (normal creatinine levels, kidney weight ratio, together with alkaline urine pH) and beneficial effects on animal health (weight gain, normal body temperature and absence of mortality).

We evaluated renal functions by measuring creatinine levels, kidney weight ratio and pH of urine and blood. A similar normalization of renal function is observed in the cisplatin model following administration of the antioxidant resveratrol [9]. Indeed, creatinine levels (Fig. 3) and kidney weight ratio (Table 1) in our control group were similar to creatinine levels (0.4 mg/dL) and kidney weight ratio (0.75%) observed in the control group in this latter study [9]. In our study, $NaHCO_3$ pretreatment prior to cisplatin was associated with normal kidney weight ratios (Table 1) and creatinine levels (Fig. 3). Following resveratrol/cisplatin treatment, normal creatinine levels (0.42 mg/dL) and kidney weight ratios (0.73%) are also observed [9]. Moreover, in our study, pretreatment with 4% sodium bicarbonate prior to

cisplatin produced an alkaline urine pH (8.11 and 7.84 at 16 and 28 days, respectively), but normal blood pH (Table 1)), consistent with clinical findings [21]. Indeed, an alkali pH (above 7.5) was observed in all (n=26) patients pre-treated with 8.4% sodium bicarbonate before methotrexate treatment [21]. By comparison, pre-treatment with saline, a pretreatment commonly used in animal studies of cisplatin-induced neuropathy, was associated with an acidic urine pH (6.62 (3 cisplatin cycles over 16 days) and 6.36 (4 cisplatin cycles over 28 days) respectively) but normal blood pH (Table 1), higher kidney weight ratios (1.11 and 1.01% for 16 and 28 days, respectively) and higher creatinine levels (0.92 and 0.98 mg/dL for 16 and 28 days, respectively). Creatinine levels (1.22 mg/dL) and kidney weight ratio (1.06%) were increased by cisplatin treatment in the resveratrol study, an effect confirmed and corroborated by our findings [9].

Ketones (ketone levels (0.5–3.00 mg/dL)) are the end products of fatty acid metabolism and high ketone levels are indicative of keto-acidosis, a serious condition characterized by high acidity of bodily fluids [3,13,17]. Interestingly, ketone levels in saline/cisplatin-treated rats are increased in comparison with those observed in control/saline or $NaHCO_3$/cisplatin-treated rats. The increase in ketone levels in saline/cisplatin-treated rats suggests that these rats could develop keto-acidosis if treated for a longer period of time [3,13]. However, glucose levels were not affected by saline/cisplatin treatment. In fact, glucose levels (50 to 135 mg/dL)) were similar between all treatment groups (see Fig. 3) [13,23]. Thus, cisplatin-induced neuropathy is not associated with high levels of glucose observed in models of diabetic neuropathy [28].

Beneficial properties of $NaHCO_3$ pretreatment are demonstrated by improved animal health, increase in weight gain (Fig.1A,B), normal body temperature (Fig.1C,D) and absence of mortality in each cisplatin dosing paradigm. However, saline/cisplatin-treated groups exhibited failure of weight gain, lower body temperature and mortality ranging from 11 to 20% following 3 or 4 once weekly cycles of cisplatin (Fig.1A–D). Studies of renal functions altered by cisplatin do not typically report general health of animals [2,9]. To our knowledge, preclinical studies have not previously used alkalis to buffer acidic effects of cisplatin and improve animal health. Importantly, cisplatin-induced mechanical and cold allodynia was similar in the presence or absence of $NaHCO_3$. Thus, the present model is particularly useful for maintaining animal health status in long term studies of cisplatin-induced neuropathy. More work is necessary to demonstrate the impact of sodium bicarbonate pretreatment on anti-tumor effects of cisplatin.

Subcutaneous saline has been represented to ameliorate cisplatin-induced nephrotoxicity [5,6]. However, this approach did not increase weight gain and was associated with mortality, especially when high (i.e. 3 mg/kg i.p.) cisplatin doses are employed [6,27]. Indeed, mortality rates of 50% have been reported with this saline/cisplatin dosing paradigm [27]. It is thus possible that saline hyperhydratation can only be achieved with more frequent saline injections (e.g. 3–4 times per day). However, saline lacks the alkaline properties of $NaHCO_3$ which are beneficial in an acidic environment.

Other treatments may also be effective in preventing toxic effects of cisplatin. Indeed, antioxidants (e.g. resveratrol, vitamin C) given before or after cisplatin administration may prevent or decrease nephrotoxicity in animals [2,9]. The hypolipidemic drug simvastatin has also been reported to attenuate cisplatin-induced kidney damage [14]. The combination of our approach with an antioxidant and/or simvastatin could potentially produce synergistic beneficial effects that prevent or reduce cisplatin-induced renal toxicity.

In conclusion, our studies provide direct evidence that once weekly injection of sodium bicarbonate prior to cisplatin treatment produces a beneficial impact on animal health (i.e.

normal weight gain, no change in body temperature and absence of mortality over 28 days). The lowered body temperature observed in saline/cisplatin-treated groups confirms previous reports of cisplatin-induced hypothermia in dosing paradigms that lacked sodium bicarbonate pretreatment [5,13]. Beneficial changes in renal functions (decrease in creatinine levels, normal kidney weight ratio and higher urine pH) were observed in the NaHCO$_3$/cisplatin group relative to saline/cisplatin treated groups at each survival time. In fact, sodium bicarbonate pretreatment normalized these parameters to that observed in control groups that did not receive cisplatin. Sodium bicarbonate pretreatment also failed to alter the development of cisplatin-induced mechanical or cold allodynia. This method constitutes a valuable approach to improve general health of cisplatin-treated rodents and may also facilitate future research employing long term evaluations of chemotherapy-induced toxicities such as peripheral neuropathy.

Acknowledgments

Abbreviations

ANOVA	analysis of variance
BL	baseline
NaCl	sodium chloride
NaHCO$_3$	sodium bicarbonate
inj	injection
i.p.	intraperitoneal
s.c.	subcutaneous

References

1. Alberts DS, Noel JK. Cisplatin-associated neurotoxicity: can it be prevented? Anticancer Drugs. 1995; 6:369–383. [PubMed: 7670134]

2. Antunes LM, Darin JD, Bianchi MD. Protective effects of vitamin c against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. Pharmacol. Res. 2000; 41:405–411. [PubMed: 10704263]

3. Askew EW, Dohm GL, Huston RL. Fatty acid and ketone body metabolism in the rat: response to diet and exercise. J. Nutri. 1975; 105:1422–1432.

4. Authier N, Balayssac D, Marchand F, Ling B, Zangarelli A, Descoeur J, Coudore F, Bourinet E, Eschalier A. Animal models of chemotherapy-evoked painful peripheral neuropathies. Neurotherapeutics. 2009; 6:620–629. [PubMed: 19789067]

5. Authier N, Fialip J, Eschalier A, Coudoré F. Assessment of allodynia and hyperalgesia after cisplatin administration to rats. Neurosci. Lett. 2000; 291:73–76. [PubMed: 10978577]

6. Authier N, Gillet JP, Fialip J, Eschalier A, Coudore F. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Exp. Neurol. 2003; 182:12–20. [PubMed: 12821373]

7. Bacci G, Ferrari S, Longhi A, Forni C, Loro L, Beghelli C, Tremosini M, Versari M. Delayed methotrexate clearance in osteosarcoma patients treated with multiagent regimens of neoadjuvant chemotherapy. Oncol. Rep. 2003; 10:851–857. [PubMed: 12792734]

8. Boyiadzis, MM.; Lebowitz, PF.; Frame, JN.; Fojo, T. Hematology-Oncology Therapy. New York: McGraw Hill, Medical Publishing Division; 2007.

9. Do Amaral CL, Francescato HD, Coimbra TM, Costa RS, Darin JD, Antunes LM, Bianchi MdeL. Resveratrol attenuates cisplatin-induced nephrotoxicity in rats. Arch. Toxicol. 2008; 82:363–370. [PubMed: 18026934]

10. Elwell MR, Sammons ML, Liu CT, Beisel WR. Changes in blood pH in rats after infection with Streptococcus pneumoniae. Infect. Immun. 1975; 11:724–726. [PubMed: 235492]

11. Guindon J, Lai Y, Takacs SM, Bradshaw HB, Hohmann AG. Alterations in endocannabinoid tone following chemotherapy-induced peripheral neuropathy: Effects of endocannabinoid deactivation inhibitors targeting fatty-acid amide hydrolase and monoacylglycerol lipase in comparison to reference analgesics following cisplatin treatment. Pharmacol. Res. 2013; 67:94–109. [PubMed: 23127915]

12. Haylor J, Lote CJ. Urine pH and the relationship between urine flow and urinary prostaglandin E excretion in the rat. J. Endocrinol. 1986; 108:247–253. [PubMed: 3950529]

13. Hillyer, EV.; Quesenberry, KE. Ferrets, rabbits and rodents; clinical medicine and surgery. First ed.. New York: Elsevier Health Science; 1996.

14. I eri S, Ercan F, Gedik N, Yüksel M, Alican I. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. Toxicology. 2007; 230:256–264. [PubMed: 17196726]

15. Keppler A, Gretz N, Schimdt R, Kloetzer HM, Groene HJ, Lelongt B, Meyer M, Sadick M, Pill J. Plasma creatinine determination in mice and rats: an enzymatic method compares favorably with a high-performance liquid chromatography assay. Kidney Int. 2007; 71:74–78. [PubMed: 17082757]

16. Kishore BK, Krane CM, Di Iulio D, Menon AG, Cacini W. Expression of renal aquaporins 1, 2, and 3 in a rat model of cisplatin-induced polyuria. Kidney Int. 2000; 58:701–711. [PubMed: 10916093]

17. Koch DD, Feldbruegge DH. Optimized kinetic method for automated determination of beta-hydroxybutyrate. Clin. Chem. 1987; 33:1761–1766. [PubMed: 3665027]

18. Liu M, Chien C, Burne-Taney M, Molls RR, Racusen LC, Colvin RB, Rabb H. A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. J. Am. Soc. Nephrol. 2006; 17:765–774. [PubMed: 16481417]

19. McCarty MF, Whitaker J. Manipulating tumor acidification as a cancer treatment strategy. Altern. Med. Rev. 2010; 15:264–272. [PubMed: 21155627]

20. Perry, MC. Companion Handbook to the Chemotherapy Sourcebook. Baltimore: Williams & Wilkins; 1999.

21. Mir O, Ropert S, Babinet A, Alexandre J, Larousserie F, Durand JP, Enkaoua E, Anract P, Goldwasser F. Hyper-alkalinization without hyper-hydration for the prevention of high-dose methotrexate acute nephrotoxicity in patients with osteosarcoma. Cancer Chemother. Pharmacol. 2010; 66:1059–1063. [PubMed: 20155268]

22. Sánchez-González PD, López-Hernández FJ, López-Novoa JM, Morales AI. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. Crit. Rev. Toxicol. 2011; 41:803–821. [PubMed: 21838551]

23. Schroeder HA. Serum cholesterol and glucose levels in rats fed refined and less refined sugars and chromium. J. Nutri. 1969; 97:237–242.

24. Sean Eardley K, Cockwell P. Macrophages and progressive tubulointerstitial disease. Kidney Int. 2005; 68:437–455. [PubMed: 16014021]

25. Silva AS, Yunes JA, Gillies RJ, Gatenby RA. The potential role of systemic buffers in reducing intratumoral extracellular pH and acid-mediated invasion. Cancer Res. 2009; 69:2677–2684. [PubMed: 19276380]

26. Vera G, Chiarlone A, Cabezos PA, Pascual D, Martín MI, Abalo R. WIN 55,212-2 prevents mechanical allodynia but not alterations in feeding behaviour induced by chronic cisplatin in the rat. Life Sci. 2007; 81:468–479. [PubMed: 17673260]

27. Vera G, Chiarlone A, Martín MI, Abalo R. Altered feeding behaviour induced by long-term cisplatin in rats. Auton. Neurosci. 2006; 126–127:81–92.

28. Wiffen PJ. Enhanced glucose control for preventing and treating diabetic neuropathy. J. Pain Palliat. Care Pharmacother. 2012; 26:380.

29. Wong P, Lee C, Tannock IF. Reduction of intracellular pH as a strategy to enhance the pH-dependent cytotoxic effects of melphalan for human breast cancer cells. Clin. Cancer Res. 2005; 11:3553–3557. [PubMed: 15867259]

30. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. Am. J. Med. Sci. 2007; 334:115–124. [PubMed: 17700201]

31. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16:109–110. [PubMed: 6877845]

Highlights

NaHCO$_3$ simple method to reduce adverse renal effects of repeated cisplatin treatment

NaHCO$_3$/cisplatin show weight gain, normal temperature, creatinine and kidney ratio

NaCl/cisplatin show lower weight and temperature, higher creatinine and kidney ratio

Pretreatment with either NaCl or NaHCO$_3$ does not prevent cisplatin-induced neuropathy

NaHCO$_3$ pretreatment induce good health and normal creatinine levels and kidney ratio
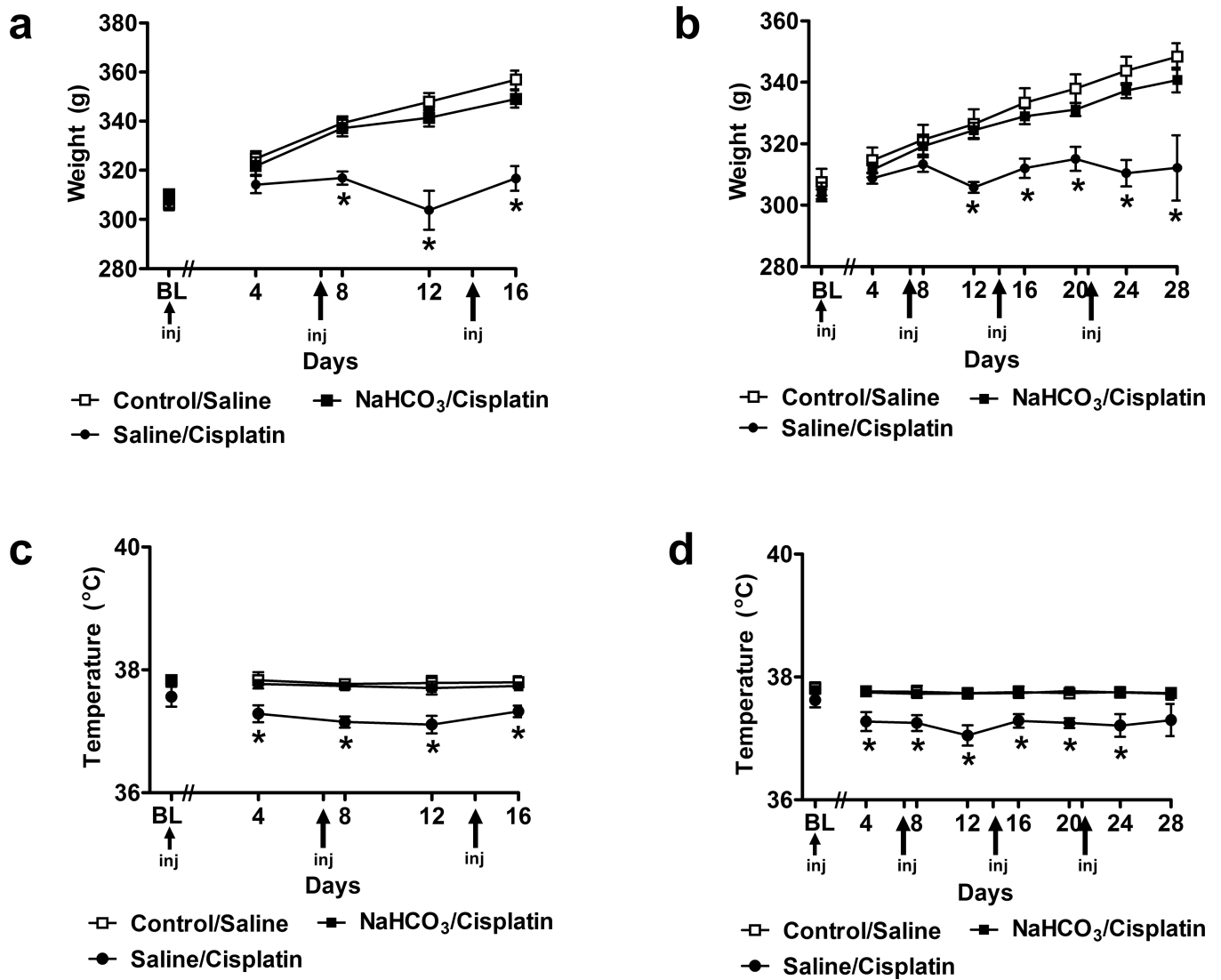
Fig. 1.
Pretreatment with 4% sodium bicarbonate ($NaHCO_3$) prior to cisplatin ($NaHCO_3$/cisplatin) prevented changes in body weight (g) and body temperature (°C) observed in saline/cisplatin-treated groups relative to control/saline groups. Animals received 3 or 4 once weekly injections of cisplatin over (a,c) 16 or (b,d) 28 days, respectively. Data are expressed as mean ± s.e.m. (n = 8–44 per group). * $P < 0.045$ vs. control/saline or $NaHCO_3$/cisplatin groups.
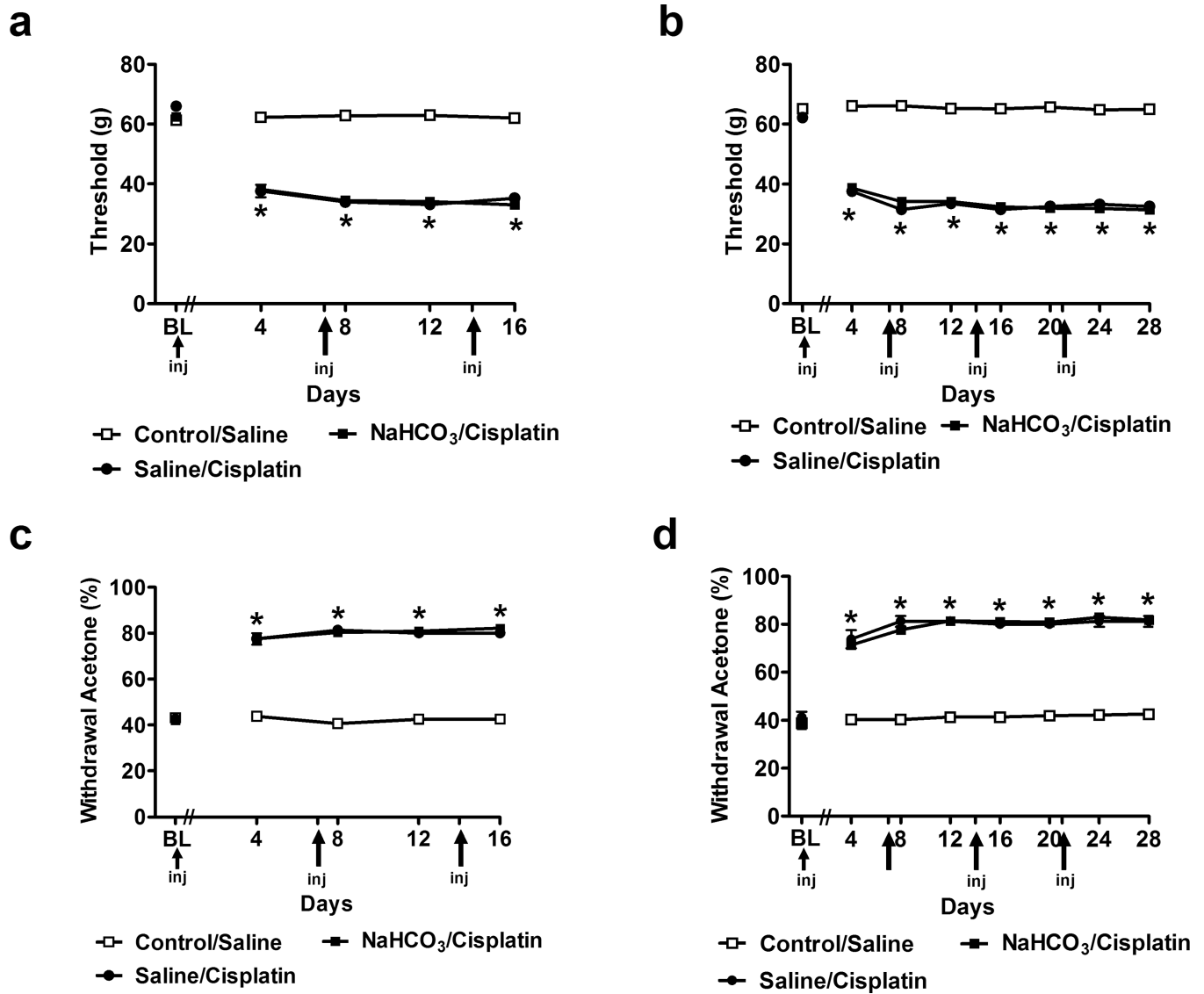
Fig. 2.
Cisplatin produces time-dependent behavioral sensitization to mechanical and cold stimulation. Time course of development of (a,b) mechanical and (c,d) cold allodynia in NaHCO$_3$/cisplatin or saline/cisplatin-treated groups relative to control/saline-treated control group. Groups received 3 (a,c) or 4 (b,d) once weekly injections and were monitored for 16 or 28 days, respectively. Data are expressed as mean ± s.e.m. (n = 8–44 per group). * $P<$ 0.0001 vs. control/saline group.
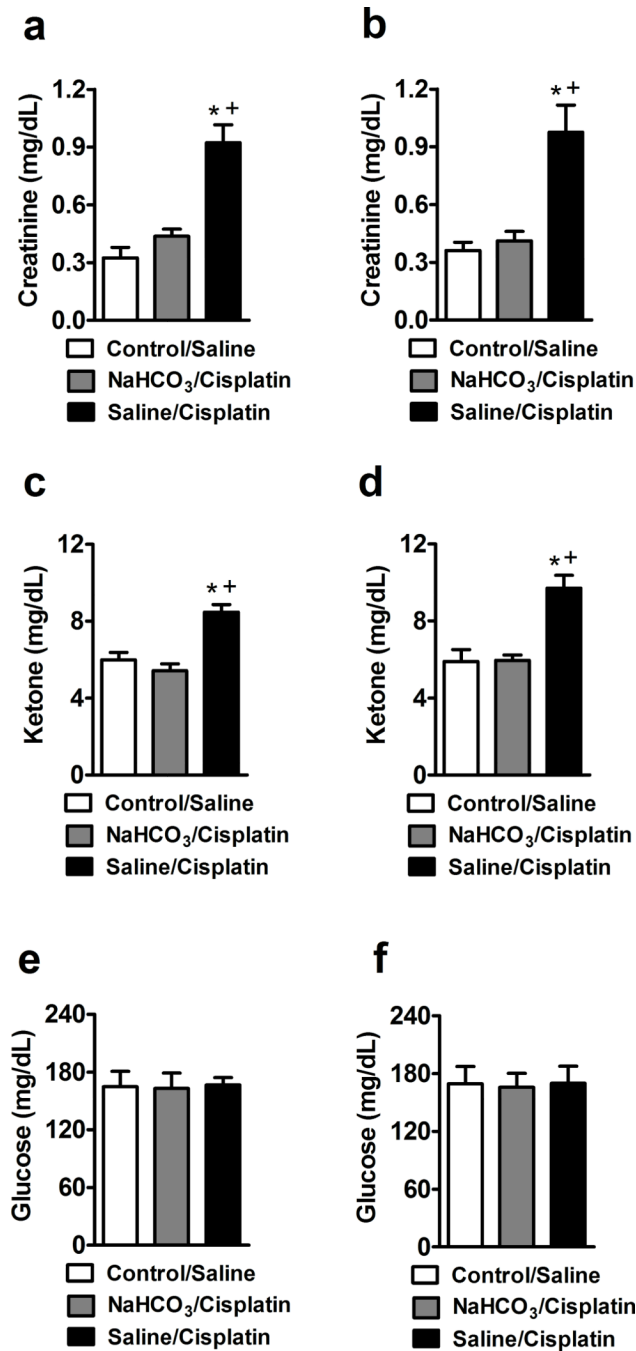
Fig. 3.
Creatinine (a,b) and ketone (c,d) levels are increased in saline/cisplatin relative to control/
saline or NaHCO$_3$/cisplatin-treated groups. Animals received 3 or 4 once weekly injections
over 16 (a,c,e) or 28 (b,d,f) days. Glucose levels did not differ in any group in either dosing
paradigm (e,f). Data are expressed as mean ± s.e.m. (n = 8–9 per group). * $P < 0.0001$ vs.
control/saline group; + $P < 0.0001$ vs NaHCO$_3$/Cisplatin group.

Table 1

Effect of NaHCO$_3$ or saline pretreatment on urine pH, blood pH and kidney weight ratio in cisplatin and saline-treated rats

		Urine PH	Blood PH (renal artery)	Kidney weight ratio (%)
Control/Saline	16 Days	6.95 ± 0.04	7.42 ± 0.03	0.77 ± 0.01
	28 Days	7.09 ± 0.07	7.37 ± 0.05	0.72 ± 0.02
NaHCO$_3$/Cisplatin	16 Days	*8.11 ± 0.07	7.45 ± 0.08	0.80 ± 0.01
	28 Days	*7.84 ± 0.11	7.36 ± 0.03	0.78 ± 0.02
Saline/Cisplatin	16 Days	6.62 ± 0.25	7.39 ± 0.04	#T1.11 ± 0.08
	28 Days	+6.36 ± 0.17	7.24 ± 0.05	#T1.01 ± 0.09

Data are expressed as mean ± s.e.m. (n = 8–9 per group) of measurements determined 16 or 28 days following initiation of cisplatin/saline dosing.

*P< 0.001 vs. control/saline or saline/cispaltin groups;

+P< 0.001 vs control/saline group;

#P< 0.002 vs. control/saline or NaHCO$_3$/csipaltin groups;

TP< 0.016 vs. NaHCO$_3$/cispaltin group.