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

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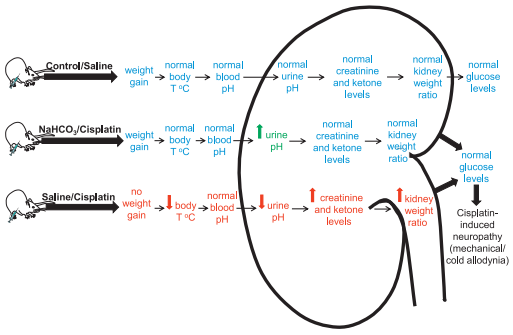
HIGHLIGHTS

- $NaHCO_3$ simple method to reduce adverse renal effects of repeated cisplatin treatment.
- $NaHCO_3$/cisplatin show weight gain, normal temperature, creatinine and kidney ratio.
- Saline/cisplatin show lower weight and temperature, higher creatinine and kidney ratio.
- Pretreatment with either Saline or $NaHCO_3$ does not prevent cisplatin-induced neuropathy.
- $NaHCO_3$ pretreatment induce good health and normal creatinine levels and kidney ratio.

GRAPHICAL ABSTRACT



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ABSTRACT

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 minimizes 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 to 50% [6,27]. Attempts to minimize mortality in rodents involve reductions in frequency, duration and/or dosing of cisplatin [4 for

Abbreviations: ANOVA, analysis of variance; BL, baseline; NaCl, sodium chloride; $NaHCO_3$, sodium bicarbonate; inj, injection; i.p., intraperitoneal; s.c., subcutaneous.

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a review]. 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 12 h light (7 am–7 pm)/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 was 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 m (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 ($F_{2,45} = 15.35$, $P < 0.0001$; days 4–16 ($P < 0.011$); Fig. 1C) and ($F_{2,81} = 12.21$, $P < 0.0001$; days 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 and 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 and 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 and 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 and D) levels in whole blood relative