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SHORT COMMUNICATION

N-ACETYLCYSTEINE PREVENTS BUT DOES NOT REVERSE DEXAMETHASONE-INDUCED HYPERTENSION

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SUMMARY

1. We have shown previously that *N*-acetylcysteine (NAC) prevents the increase in blood pressure induced by adrenocorticotropin treatment. The present study investigated the effect of NAC on dexamethasone (Dex)-induced hypertension.

2. Male Sprague-Dawley rats were randomly divided into six groups ($n = 10$ in each). In a prevention study, NAC (10 g/L in the drinking water) was given for 4 days prior to and 11 days during concurrent treatment with saline (0.1 mL/rat per day) or with Dex (10 μg/rat per day). In a reversal study, daily injections of Dex or saline began 8 days before NAC and cotreatment continued for 5 days. Systolic blood pressure (SBP) was measured on alternate days using a tail-cuff system.

3. Dexamethasone significantly increased SBP from 113 ± 4 to 139 ± 6 mmHg ($n = 10$; $P < 0.01$). *N*-Acetylcysteine alone had no effect on SBP. In NAC + Dex-treated rats, SBP was significantly lower than that of Dex-treated rats ($P' < 0.01$). In fully established Dex-hypertension NAC was ineffective and SBP remained high.

4. Both Dex and NAC treatments decreased bodyweight gain. *N*-Acetylcysteine reduced food and water consumption. Dexamethasone reduced thymus weight ($P' < 0.01$) but NAC treatment did not alter this marker of glucocorticoid activity.

5. Dexamethasone tended to decrease plasma NO_x, whereas NAC restored plasma NO_x concentrations to control levels. *N*-Acetylcysteine had no effect on Dex-induced increased plasma F_2-isoprostane concentrations.

6. In conclusion, NAC partially prevented, but did not reverse, Dex-induced hypertension.

Key words: *N*-acetylcysteine, dexamethasone, hypertension, oxidative stress.

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INTRODUCTION

The synthetic glucocorticoid dexamethasone (Dex) is used as an anti-inflammatory or immunosuppressant agent in the treatment of a variety of diseases. Chronic Dex treatment induces hypertension in rats[1,2] and humans.[3] Administration of Dex increased systolic and mean arterial pressure and decreased serum nitrogen intermediate (NO_x) concentrations in wild-type mice, but not endothelial nitric oxide synthase (eNOS)-nockout mice. In addition, Dex treatment decreased eNOS mRNA levels in wild-type mouse heart, liver and kidney.[4] *In vitro* studies have shown that Dex downregulates both eNOS[5] and inducible nitric oxide synthase (iNOS) expression,[6] and inhibits nitric oxide synthase (NOS) precursor L-arginine transport and NOS cofactor tetrahydrobiopterin (BH_4) synthesis. The inhibition of eNOS mRNA expression by Dex was prevented by the glucocorticoid receptor antagonist RU486.[5] These results suggest that Dex-induced hypertension is associated with decreased nitric oxide (NO) production. However, BH_4 supplementation did not prevent Dex-induced hypertension.[7]

Reactive oxygen species are scavengers for NO. Dexamethasone-induced hypertension is associated with increased oxidative stress, measured by plasma F_2-isprostane analysis in rats,[1] and is prevented by both the anti-oxidant tempol and the NADPH oxidase inhibitor apocynin.[1,2] Thus, an NO–redox imbalance plays an important role in this model of hypertension. However, neither tempol nor apocynin are available for use in humans.

N-Acetylcysteine (NAC) is a widely studied water-soluble antioxidant that is in clinical use as a mucolytic agent, an antidote for paracetamol (paracetamol) overdose and for ischaemia–reperfusion injury.[8,9] We have demonstrated previously that NAC (10 g/L in drinking water) prevents but does not reverse adrenocorticotropic hormone (ACTH)-induced hypertension in rats.[10] However, L-arginine, the substrate for NOS, prevents and reverses ACTH-[11,12] but not Dex-induced[2,13] hypertension, implying there is difference in the mechanism of natural and synthetic glucocorticoid (GC)-induced hypertension. The aim of the present study was to investigate whether NAC could prevent and/or reverse Dex-induced hypertension in the rats.

METHODS

Animals

This project was approved by the Animal Experimental Ethics Committee of the Australian National University (Protocol number J.HB.27.07). Male Sprague-Dawley rats (bodyweight 200 g; Animal Resources Centre, Perth, WA, Australia) were housed in plastic cages at a constant temperature of 20–22°C and under a 12 h light–dark cycle. Rat had access to rat chow and tap water *ad libitum*. Rats were handled and acclimatized to the equipment for 2 weeks prior to the experiments.

Protocol

In the prevention study, NAC (10 g/L in the drinking water; Sigma, St Louis, MO, USA) was given for 4 days prior to and 11 days during concurrent treatment with saline (0.1 mL/rat per day) or Dex (10 μg/rat per day, s.c.; David Bull Laboratories, Mulgrave, Vic., Australia). In the reversal study, daily injections of Dex or saline began 8 days before NAC was coadministered for a further 5 days. Rats were randomly assigned to one of the following groups ($n = 10$ in each group): (i) Group 1, saline; (ii) Group 2, Dex; (iii) Group 3, NAC + saline prevention; (iv), Group 4, NAC + Dex prevention; (v), Group 5, saline + NAC reversal; and (vi) Group 6, Dex + saline reversal.

Systolic blood pressure measurements

Systolic blood pressure (SBP) was measured at 13.00–15.00 hours on alternate days before injection or drug administration using a tail-cuff system (Narco Biosystems, Houston, TX, USA).[10]

Metabolic measurement

Bodyweight (BW) was assessed on alternate days and food and water consumptions were measured daily. Thymus wet weight was expressed as mg/100 g BW.

Plasma F_2-isoprostane and NO_x concentrations

Blood was collected via a right ventricular puncture in tubes containing EDTA + glutathione (for the F_2-isoprostane assay) and citrate (for the NO_x assay) from rats under anaesthesia and centrifuged at 1500 *g* for 15 min at 4°C, before plasma was collected. Plasma F_2-isoprostanes were measured using a previously described method.[14] Plasma NO_x was determined by the reduction of nitrate to nitrite using a commercial kit (Total Nitric Oxide Assay Kit; Pierce Endogen, Rockford, IL, USA), as described previously.[10]

Statistical analysis

Data are expressed as the mean±SEM and were analysed using SPSS (version 14.0; SPSS, Chicago, IL, USA) by Student's *t*-test and repeated-measures ANOVA. Greenhouse-Geisser $P < 0.05$ and P' (Bonferroni-corrected value) ≤ 0.05 were regarded as significant.

RESULTS

Systolic blood pressure

Systolic blood pressure in Dex-treated rats was higher than in saline-treated rats ($P' < 0.01$; Fig. 1a). *N*-Acetylcysteine alone had no effect on SBP. In the prevention study, SBP in NAC + Dex-treated rats was significantly lower than that of Dex-treated rats ($P' < 0.01$), but still higher than NAC + saline-treated rats ($P' < 0.05$; Fig. 1a). In the reversal study, NAC failed to significantly reduce the increase in SBP produced by Dex (SBP 138 ± 2 and 139 ± 6 mmHg in the Dex + NAC and Dex alone groups, respectively).

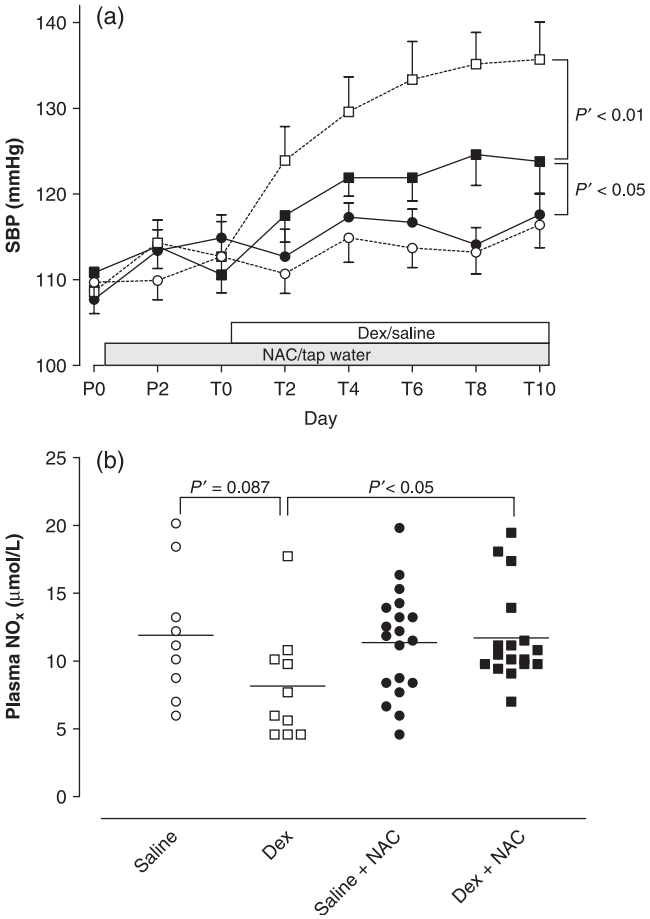

Fig. 1 (a) Systolic blood pressure (SBP) in the prevention study: saline ($n = 10$; ○), dexamethasone (Dex; $n = 10$; □), *N*-acetylcysteine (NAC) + saline prevention ($n = 10$; ●), NAC + Dex prevention ($n = 10$; ■). P0, P2, NAC pretreatment days; T0, T2 . . . T10, NAC + Dex/saline treatment days. (b) Plasma NO_x concentrations: saline ($n = 10$; ○); Dex ($n = 10$; □); NAC + saline ($n = 20$; ●); NAC + Dex ($n = 20$; ■).

Metabolic measurements

Bodyweight gain was decreased in both Dex (from 244 ± 5 to 277 ± 6 g) and NAC (from 265 ± 4 to 287 ± 5 g) groups compared with saline (from 248 ± 5 to 294 ± 5 g; both $P' < 0.01$). *N*-Acetylcysteine reduced water and food consumption (21 ± 0.4 mL and 20 ± 0.2 g, respectively; both $P' < 0.01$) compared with saline treatment (36 ± 0.6 mL and 22 ± 0.2 g, respectively). Dexamethasone reduced thymus weight (46 ± 3 mg/100 g BW) compared with saline-treated rats (146 ± 10 mg/100 g BW; $P' < 0.01$). *N*-Acetylcysteine treatment did not alter this marker of GC activity (136 ± 6 and 51 ± 3 mg/100 g BW in the NAC + saline and NAC + Dex groups, respectively).

Plasma F_2-isoprostane concentrations

Plasma F_2-isoprostane concentrations were higher in Dex-treated rats (10.9 ± 1.2 pmol/L) compared with saline-treated rats (8.0 ± 0.6 pmol/L; $P' = 0.05$). In the prevention study, plasma F_2-isoprostane concentrations were lower with NAC + saline treatment

(6.3 ± 0.2 pmol/L) compared with saline-treated rats ($P' = 0.046$). However, NAC had no effect on Dex-induced increases in plasma F_2-isoprostane concentrations (10.1 ± 0.6 pmol/L).

Plasma NO_x concentrations

Dexamethasone treatment tended to decrease plasma NO_x, whereas NAC treatment restored plasma NO_x concentrations to those seen in saline controls (Fig. 1b).

DISCUSSION

In the present study, Dex (10 μg/rat per day, s.c.) increased SBP and reduced the thymus weight and rate of bodyweight gain, consistent with previous studies.[15,16] The major finding was that NAC (10 g/L in drinking water; 0.82 ± 0.01 g/kg per day) partially prevented, but did not reverse, Dex-induced hypertension. This result is consistent with the effect of NAC on ACTH-induced hypertension.[10] Because NAC did not affect the SBP of normotensive rats, it is unlikely to have a direct vasodilatory effect. *N*-Acetylcysteine had no effect on thymus weight, indicating that it does not affect GC activity. The anti-oxidant properties of NAC, such as restoration of intracellular glutathione levels,[17,18] direct hydroxyl radical scavenging[19,20] and reduced lipid peroxidation,[19] may contribute to its antihypertensive effect. In the present study, we confirmed that Dex-induced hypertension is accompanied by increased plasma F_2-isoprostane concentrations,[1] a marker of systemic oxidative stress and decreased plasma NO_x concentrations.[13] Although NAC failed to reduce Dex-induced increases in plasma F_2-isoprostane concentrations, it restored Dex-induced decreases in plasma NO_x levels, an indication of an improved NO–redox imbalance.

Daily water consumption was decreased in all NAC-treated rats (in both prevention and reversal studies). However, NAC only affected the development of hypertension and had no effect on established ACTH-[10] or Dex-induced hypertension. Therefore, it is unlikely that the NAC-induced decrease in blood pressure occurs via reduced plasma volume. *N*-Acetylcysteine (20 g/L in drinking water for 8 weeks) partially prevented the rise in blood pressure in young (developing hypertension), but not old (established hypertension), spontaneously hypertensive rats (SHR).[21] The anti-oxidant action of NAC (measured as a decrease in the concentration of conjugated dienes or inhibition of nuclear factor-κB expression) was greater in young than adult SHR.[21] Similarly, eNOS protein expression was attenuated more in young than in adult SHR, although NAC treatment increased NOS activity to a similar extent in both young and adult rats.[21] The effect of NAC on established GC-induced hypertension is similar to findings in old SHR.[21]

In conclusion: (i) NAC partially prevented the development of Dex-induced hypertension in male Sprague-Dawley rats; (ii) NAC did not reverse established Dex-induced hypertension; and (iii) NAC restored Dex-induced decreases in plasma NO_x concentrations.

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