**SHORT COMMUNICATION**

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

Susanne Krug,* Yi Zhang,* Trevor A Mori,† Kevin D Croft,† Janine J Vickers,* Leanne K Langton* and Judith A Whitworth*

*The High Blood Pressure Research Unit, The John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory and †School of Medicine and Pharmacology, University of Western Australia and The Cardiovascular Research Centre, Perth, Western Australia, Australia

**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 NOx, whereas NAC restored plasma NOx concentrations to control levels. N-Acetylcysteine had no effect on Dex-induced increased plasma F2-isoprostane concentrations.

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

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

**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 and humans. Administration of Dex increased systolic and mean arterial pressure and decreased serum nitrogen intermediate (NOx) 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. In vitro studies have shown that Dex downregulates both eNOS and inducible nitric oxide synthase (iNOS) expression, and inhibits nitric oxide synthase (NOS) precursor L-arginine transport and NOS cofactor tetrahydrobiopterin (BH4) synthesis. The inhibition of eNOS mRNA expression by Dex was prevented by the glucocorticoid receptor antagonist RU486. These results suggest that Dex-induced hypertension is associated with decreased nitric oxide (NO) production. However, BH4 supplementation did not prevent Dex-induced hypertension.

Reactive oxygen species are scavengers for NO. Dexamethasone-induced hypertension is associated with increased oxidative stress, measured by plasma F2-isoprostane analysis in rats, and is prevented by both the anti-oxidant tempol and the NADPH oxidase inhibitor apocynin. 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. We have demonstrated previously that NAC (10 g/L in drinking water) prevents but does not reverse adrenocorticotropic hormone (ACTH)-induced hypertension in rats. However, L-arginine, the substrate for NOS, prevents and reverses ACTH 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 JHB.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). 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. 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).

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 salinetreated 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 F₂-isoprostane concentrations

Plasma F₂-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 F₂-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. 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. 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, direct hydroxyl radical scavenging and reduced lipid peroxidation, 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, a marker of systemic oxidative stress and decreased plasma NO$_x$ concentrations. 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 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), although NAC treatment increased NO levels in young SHR. 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. The effect of NAC on established GC-induced hypertension is similar to findings in old SHR.

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.

**ACKNOWLEDGEMENTS**

This study was supported by a National Health and Medical Research Council of Australia project grant (ID 418026). The authors thank Matthew Sutton and Yew K Tan for their technical assistance.