Aspirin Prevents and Partially Reverses Adrenocorticotropic Hormone-Induced Hypertension in the Rat

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Background: Glucocorticoid-induced hypertension is associated with increased oxidative stress. The aim of the present study was to investigate the effects of aspirin, a potent antioxidant, on adrenocorticotropic hormone (ACTH) and dexamethasone (Dex)-induced hypertension.

Methods: Male Sprague-Dawley (SD) rats were treated with saline, ACTH (0.2 mg/kg/d subcutaneously) or Dex (10 μg/rat/d subcutaneously). Aspirin (100 mg/kg/d in drinking water) was given 4 days before and during glucocorticoid-treatment (prevention studies). In reversal studies, saline, ACTH, or Dex was administered for 13 days and at day 8 (T8), rats were co-administered aspirin for 5 days. Systolic blood pressure (BP) was measured by the tail-cuff method. Thymus wet weight was measured as a marker of glucocorticoid activity and lucigenin-enhanced chemiluminescence as a marker of aortic superoxide production.

Results: Saline or aspirin alone did not change systolic BP. Systolic BP was increased by ACTH (mean ± SEM; from 99 ± 2 to 133 ± 4 mm Hg, n = 10, P < .001) and Dex (from 102 ± 3 to 125 ± 5 mm Hg, n = 10, P < .001). Aspirin prevented the development of hypertension caused by ACTH (P′ < .01) and tended to prevent Dex-induced hypertension (P′ = .07). ACTH-but not Dex-induced hypertension was partially reversed by aspirin. Both ACTH and Dex decreased thymus weight. Aspirin had no effect on thymus weight. ACTH tended to increase lucigenin-enhanced chemiluminescence (P′ = .07). Aspirin had no effect on this marker of tissue superoxide production.


Key Words: Aspirin, glucocorticoid, hypertension, superoxide.

Renal nitric oxide (NO) synthase (iNOS and eNOS) expression is reduced in both adrenocorticotropic hormone (ACTH) and corticosterone-treated rats.1 Glucocorticoid-induced hypertension is associated with decreased plasma reactive nitrogen intermediate (NOx) concentrations,2–4 increased plasma F2-isoprostane concentrations (a marker for oxidative stress),5,6 and lucigenin-enhanced chemiluminescence in the aorta (a marker of tissue superoxide production).7,8 The superoxide scavengers tempol and N-acetylcysteine (NAC), prevent ACTH- or dexamethasone (Dex)-induced hypertension in rats,5,6,8 indicating that reactive oxygen species (ROS) overproduction plays an important role in glucocorticoid-induced hypertension. Furthermore, the angiotensin-converting enzyme (ACE) inhibitor ramipril9 and the NADPH oxidase inhibitor apocynin7,10 prevent and reverse ACTH- or Dex-induced hypertension. These results suggest that angiotensin II stimulated superoxide production through NAD(P)H oxidase is a major source of increased ROS in ACTH- and Dex-induced hypertension in the rat, and plays a major role in glucocorticoid-induced hypertension.

Unlike apocynin, aspirin (acetylsalicylic acid) is widely used in clinical practice. It is an anti-inflammatory and cardiovascular protective drug with potent inhibitory effects on cyclooxygenases. The cardiovascular beneficial effects cannot be explained fully by its antiplatelet aggregation properties, as other platelet inhibitors have not produced the same cardiovascular beneficial effects.11 Aspirin inhibits ROS generation by cyclooxygenase.12 Aspirin (100 mg/kg/d in drinking water for 3 weeks) prevented glucose-induced hypertension and superoxide overproduction (measured by lucigenin-enhanced chemiluminescence),13 and

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aspirin (10 to 100 mg/kg/d in drinking water for 12 days) reduces aortic and cardiac production of superoxide by lowering NAD(P)H oxidase activity.\textsuperscript{13–15} However, only a high dose of aspirin (100 mg/kg/d) was shown to prevent angiotensin II-induced hypertension in rats.\textsuperscript{14,15} This dose and method of aspirin treatment was used in the current study.

The aim of the present study was to investigate the effects of aspirin on ACTH- and Dex-induced hypertension. Our hypothesis was that aspirin will prevent and reverse glucocorticoid-induced hypertension by inhibiting NAD(P)H oxidase-mediated superoxide production.

**Methods**

**Animals**

Male Sprague-Dawley (SD) rats (body weight around 200 g, \( n = 90 \)) (Animal Resources Centre, Perth, WA) were used in these studies, which were approved by the Animal Experimental Ethics Committee of the Australian National University (AEEC Protocol No J.HB. 19.04). Rats were housed at constant temperature 20° to 22°C, humidity 30% to 31% and 12-h light–dark cycle. They had free access to standard commercial feed and tap water for drinking during the entire experimental period. Rats were acclimatized for surroundings, handling, and equipment for blood pressure (BP) measurement for 1 week before experiments.

**Experimental Protocol**

Adrenocorticotropic hormone (0.2 mg/kg) (Novartis Pharmaceuticals, Sydney, Australia), Dex (20 \( \mu \)g/kg) (David Bull Laboratories, Mulgrave, Victoria, Australia), or equal volume of vehicle solution (saline 1 mL/kg) were injected subcutaneously daily. In prevention studies, aspirin (acetylsalicylic acid) (100 mg/kg/d in drinking water) (Sigma, St. Louis, MO) was given 4 days before ACTH, Dex, or saline treatment and then co-administered with ACTH, Dex, or saline treatment for an additional 11 days. In reversal studies, saline, ACTH, or Dex was administered for 8 days, before being co-administered with aspirin for an additional 5 days. Aspirin solution was prepared daily and the dose was adjusted by daily body weight and water consumption. The aspirin alone treatment days were designated as “A,” in the prevention studies and ACTH or saline treatment days as “T.” The rats were randomly assigned to one of the following groups, with 10 rats per group.

**Control Groups**

Saline treatment (\( n = 10 \)): saline daily injection for 13 days (T0 to T12) and tap water to drink throughout the study.

ACTH treatment (\( n = 10 \)): ACTH daily injection from T0 to T12 and tap water to drink throughout the study.

Dex treatment (\( n = 10 \)): Dex daily injection from T0 to T12 and tap water to drink throughout the study.

**Prevention Studies**

Aspirin + saline treatment (\( n = 10 \)): rats were treated with aspirin in drinking water for 4 days (A0 to A3) followed by saline co-treatment for 11 days (T0 to T10).

Aspirin + ACTH treatment (\( n = 10 \)): rats were treated with aspirin from A0 to A3 followed by ACTH co-treatment from T0 to T10.

Aspirin + Dex treatment (\( n = 10 \)): rats were treated with aspirin from A0 to A3 followed by Dex co-treatment from T0 to T10.

**Reversal Studies**

Saline + aspirin treatment (\( n = 10 \)): saline daily injection for 13 days (T0 to T12), tap water to drink until T7, and aspirin in drinking water from T8 to T12.

ACTH + aspirin treatment (\( n = 10 \)): ACTH injection daily from T0 to T12, tap water to drink to T7, and aspirin from T8 to T12.

Dex + aspirin treatment (\( n = 10 \)): Dex injection daily from T0 to T12, tap water to drink to T7, and aspirin from T8 to T12.

**Systolic BP and Body Weight Measurements**

Systolic BP was measured at 9 to 11 AM on alternate days using a tail–cuff system (Narco Biosystems, Houston, TX). Rats were placed into restrainers on a heated plate (39° to 40°C) for 30 to 40 min. Several systolic BP measurements were recorded from each rat and the mean of four recordings (among which the difference was no more than 10 mm Hg) was accepted as the systolic BP. We have demonstrated that telemetry and tail–cuff are compatible in detecting BP effects of ACTH in the rat.\textsuperscript{16} Body weight of rats was measured on alternate days after systolic BP measurement and before injections.

**Thymus Weight**

After sacrifice, the rat thymus was isolated and weighed as a marker of glucocorticoid activity. Thymus wet weight was expressed as mg/100 g body weight.

**Aortic Superoxide Production**

Superoxide production by thoracic aortic ring segments from rats treated with saline, ACTH, Dex, aspirin, and combinations of these drugs was measured using lucigenin (5 \( \mu \)mol/L) (Sigma) enhanced chemiluminescence, as previously described.\textsuperscript{5,8} Data were expressed as counts per second per milligram dry weight.

**Statistical Analysis**

Data were expressed as mean ± SEM. Systolic BP and body weight within and between groups were analyzed by repeated measures analysis of variance (RM-ANOVA), with the Greenhouse-Geisser (G-G) adjustment for multivariate sample asphericity (SPSS v. 12.0; SPSS Inc., Chicago, IL). Thymus weight and superoxide production between
groups were compared by unpaired Student t test. The Ryan-Holm step-down Bonferroni procedure was applied to the (G-G) adjusted P values, to control the family-wise type I error rate. The adjusted \( P' \leq .05 \) was regarded as significant.

**Results**

**Systolic BP**

Daily saline injection did not alter systolic BP (T0 98 ± 2, T12 102 ± 3 mm Hg, \( P \) = not significant [NS]), whereas ACTH (99 ± 2 to 133 ± 5 mm Hg, \( P < .001 \)) and Dex (102 ± 3 to 121 ± 4 mm Hg, \( P < .001 \)) increased systolic BP. Systolic BP was higher in ACTH- (\( P' < .01 \)) and Dex-treated rats (\( P' < .05 \)) than saline-treated rats (Fig. 1).

In prevention studies, systolic BP did not significantly change in aspirin + saline (106 ± 4 T0 to 100 ± 2 mm Hg T10, \( P = NS \)), aspirin +ACTH-treated rats (113 ± 5 to 111 ± 2 mm Hg, \( P = NS \)) or aspirin + Dex-treated rats (100 ± 3 to 112 ± 5 mm Hg, \( P = NS \)). There was no difference in systolic BP between aspirin + saline and saline alone, and aspirin + ACTH and aspirin alone-treated rats. Systolic BP was lower in rats treated with aspirin + ACTH than in rats treated with ACTH alone (\( P' < .01 \)) (Fig. 1a). The difference in systolic BP between aspirin + Dex- and Dex alone-treated rats failed to reach statistical significance after Bonferroni adjustment (\( P' = .07 \)). There was no statistically significant difference in systolic BP between aspirin + Dex- and aspirin alone-treated rats (Fig. 1b).

In reversal studies, before aspirin administration, systolic BP was constant (101 ± 2 T0 to 104 ± 2 mm Hg T6, \( P = NS \)) in the saline + aspirin-treated rats and increased in ACTH + aspirin-(100 ± 1 to 129 ± 3 mm Hg, \( P < .001 \)) and Dex + aspirin-treated rats (102 ± 3 to 122 ± 3 mm Hg, \( P < .05 \)). Co-treatment with aspirin on day 8 did not change systolic BP in saline-(103 ± 3 T8 to 106 ± 3 mm Hg T12, \( P = NS \)) and Dex-treated rats (121 ± 3 T8 to 115 ± 4 mm Hg T12, \( P = NS \)) but decreased systolic BP in ACTH-treated rats (131 ± 3 to 113 ± 4 mm Hg, \( P < .001 \)). Systolic BP was lower in ACTH + aspirin-treated rats than ACTH alone-treated rats (\( P' < .01 \)) (Fig. 2a). There was no difference in systolic BP between Dex + aspirin and aspirin alone and Dex + aspirin treatment and Dex alone-treated rats (Fig. 2b).

**Body Weight**

Body weight increased in saline-(238 ± 8 T0 to 306 ± 8 g T12, \( P < .001 \)), ACTH-(243 ± 6 to 249 ± 7 g, \( P < .05 \)), and Dex-treated rats (222 ± 4 to 238 ± 6 g, \( P < .001 \)). Body weight increase was less in ACTH-(6 ± 3 g) and Dex-(16 ± 2 g) treated rats compared with saline-treated rats (68 ± 4 g, \( P' < .01 \)). In prevention studies, body weight gain was lower in aspirin + saline-(44 ± 3 g) and
aspirin + Dex-treated rats (12 ± 4 g) compared with saline-treated rats ($P' < .01$), but higher in aspirin + ACTH-treated rats (11 ± 8 g) compared with Dex- and ACTH alone-treated rats ($P' < .01$). Aspirin had no effect on body weight in reversal studies.

**Thymus Weight**

Both ACTH (48 ± 6 mg/100 g body weight) and Dex (53 ± 3 mg/100 g body weight) significantly decreased rat thymus weight compared with saline (141 ± 9 mg/100 g body weight) ($P' < .01$). In the reversal study, thymus weight was higher in the saline + aspirin group (173 ± 9 mg/100 g body weight) compared with saline alone treatment ($P' < .05$) (Fig. 3). However, thymus weight was not increased in aspirin-treated rats in the prevention study or in ACTH + aspirin- or Dex + aspirin-treated rats in reversal study.

**Production of Superoxide in the Aorta**

The lucigenin-enhanced chemiluminescence readings of aortic segments were higher in all ACTH-treated rats (ACTH alone: 208 ± 32, aspirin + ACTH prevention: 257 ± 26, and ACTH + aspirin reversal: 245 ± 56 count/sec/mg) than saline-treated rats (saline alone: 123 ± 31, aspirin + saline prevention: 112 ± 24 and saline + aspirin reversal: 113 ± 8 count/sec/mg) ($P' = .07$, $.01$, and $.05$, respectively) (Fig. 4a). Neither Dex (Fig. 4b) nor aspirin treatment (Fig. 4) altered this marker of tissue superoxide production.

**Discussion**

The major finding of the present study is that aspirin at the dose of 100 mg/kg/d prevented and partially reversed ACTH-induced hypertension in SD rats. Although aspirin tended to prevent Dex-induced hypertension ($P = .07$), it failed to reverse Dex-induced hypertension. This study confirmed previous findings that both ACTH and Dex increase systolic BP, and the hypertension is associated with decreased body and thymus weight and increased superoxide production (ACTH-induced hypertension). Aspirin had no effect on glucocorticoid activity (thymus weight) and aortic superoxide production in either ACTH- or Dex-treated rats.

Aspirin has been used for the primary prevention of cardiovascular disease. However, the usefulness of antiplatelet therapy with aspirin in essential hypertension patients is controversial. It is recommended for primary prevention only in patients at high risk in whom BP control is excellent. In addition to the risk of hemorrhage, there is concern that aspirin may attenuate the effect...
of ACE inhibitors.\textsuperscript{17,18} In methylprednisolone (20 mg/kg/week subcutaneously for 2 weeks)-induced hypertensive male Wistar rats, aspirin (100 mg/kg/d) caused mortality in animals and produced massive cardiac necrosis and renal damage as evident from histopathology.\textsuperscript{18} However, the effect of aspirin alone on BP was not investigated in this study.\textsuperscript{18} The effect of aspirin on BP varies in different hypertension models. Aspirin prevented glucose- and angiotensin II-induced hypertension.\textsuperscript{13–15} Aspirin-treated two-kidney rats developed significantly higher BPs than vehicle-treated controls, but the BP of aspirin-treated one-kidney rats increased less after clipping than those of vehicle-treated controls.\textsuperscript{19} Blood pressure in SHR with BP more than 161 mm Hg was decreased by aspirin, whereas BP of SHR less than 160 mm Hg was increased by aspirin.\textsuperscript{20}

The present study demonstrated that aspirin (100 mg/kg/d in drinking water) prevented and partially reversed ACTH-induced hypertension in the SD rats. This result is consistent with the antihypertensive effect of aspirin demonstrated previously in other rat hypertension models. In SD rats drinking 10% glucose for 3 weeks, both systolic BP and aortic tissue superoxide production (measured by lucigenin-enhanced chemiluminescence) were increased and they were positively correlated. Aspirin (100 mg/kg/d in drinking water) completely prevented the increase in BP and superoxide production, and increased plasma superoxide dismutase activity in glucose-fed rats.\textsuperscript{13} Aspirin dose dependently reduced aortic tissue/smooth muscle cell superoxide production and NAD(P)H oxidase activity in both normotensive and angiotensin II-induced hypertensive rats.\textsuperscript{14} High dose aspirin (100 mg/kg/d in drinking water) but not low dose (10 mg/kg/d) prevented angiotensin II-induced hypertension and superoxide overproduction.\textsuperscript{14} That study also demonstrated that aspirin improved the impaired aortic relaxation response to acetylcholine and attenuated the age-dependent development of hypertension in spontaneously hypertensive rats. An additional study by the same group of investigators confirmed that concurrent treatment with aspirin (100 mg/kg/d in drinking water) in angiotensin II-infused rats completely prevented the angiotensin II-induced production of superoxide, hypertension, and cardiac hypertrophy. Losartan but not other anti-inflammatory drugs, such as salicylic acid, indomethacin, and ibuprofen, showed similar effects.\textsuperscript{15} Strong evidence of the antioxidant property of aspirin was also reported in several nonhypertensive models where aspirin lowered serum malondialdehyde levels and increased reduced serum glutathione content in hypercholesterolemic rats\textsuperscript{21} or protected bovine pulmonary artery endothelial cells from hydrogen peroxide-induced apoptosis.\textsuperscript{22}

Therefore, the antihypertensive effect of aspirin was presumably acting through its antioxidant properties, in particular, inhibition of NAD(P)H oxidase-mediated superoxide production. We have demonstrated previously that oxidative stress (measured by plasma F2-isoprostane concentrations and lucigenin-enhanced chemiluminescence in aortic tissue) is increased in ACTH-induced hypertension.\textsuperscript{5–8,10,23} The fact that both ACTH- and Dex-induced hypertension were prevented and reversed by the superoxide scavenger tempol\textsuperscript{5,6} suggests that oxidative stress plays an important role in glucocorticoid-induced hypertension. Furthermore, the ACE inhibitor ramipril\textsuperscript{9} and the NAD(P)H oxidase inhibitor apocynin\textsuperscript{7,10} prevent and reverse both ACTH- and Dex-induced hypertension. These results suggest that angiotensin II-stimulated superoxide production through NAD(P)H oxidase is a major source of increased ROS in ACTH- and Dex-induced hypertension in the rat. In the present study, however, aspirin failed to affect superoxide production (measured by lucigenin-enhanced chemiluminescence). The dose and method of administration of aspirin used in the present study was the same as in previous studies.\textsuperscript{14,15} The effect of aspirin on glucocorticoid-induced hypertension was consistent with that on angiotensin II-induced hypertension.\textsuperscript{14,15} and angiotensin II-stimulated superoxide production through NAD(P)H oxidase is a major source of increased ROS in both glucocorticoid- and angiotensin II-induced hypertension. The difference of the effect of aspirin on superoxide production measurements could be due to (1) different sensitivity from different detectors or different expression of data (cpm/mg fresh tissue\textsuperscript{14,15} versus cps/mg dry tissue in the present study); (2) small numbers and between assay variability, and (3) superoxide anion production in aorta (which may not reflect redox state in resistance vessels) may be different in this model of hypertension compared with angiotensin II-induced hypertension.

There is strong evidence that glucocorticoid-induced hypertension is associated with NO deficiency.\textsuperscript{1–4,24–26,27} Adrenocorticotropic hormone, corticosterone, and Dex reduced iNOS and eNOS expression.\textsuperscript{1,2,5} \(L\)-Arginine, a precursor of NO prevents\textsuperscript{2} and partially reverses\textsuperscript{3,4} ACTH-induced hypertension in the rat, accompanied by increases in plasma arginine and NOx concentrations. The \(L\)-arginine BP-lowering effects in ACTH-treated rats was abolished by the NOS inhibitor, \(N\)-nitro-\(L\)-arginine (NOLA),\textsuperscript{3} indicating that \(L\)-arginine is likely to be working through NO generation. Lipopolysaccharide, which stimulates iNOS,\textsuperscript{26} and the NO donors isosorbide dinitrate\textsuperscript{27} and (Z)-1-\(N\)-(2-aminoethyl)-\(N\)-(2-ammonioethyl)amino)diazen-1-ium-1,2-diolate (DETA/NO)\textsuperscript{28} reversed ACTH-induced hypertension in rodents. Glucocorticoid-induced hypertension is associated with decreased plasma NOx concentrations in rats,\textsuperscript{2,4} mice,\textsuperscript{29} and humans.\textsuperscript{30} Furthermore, we have shown that agents that prevent or reverse glucocorticoid-induced hypertension are either NO donors (\(L\)-arginine,\textsuperscript{2–4} lipopolysaccharide (LPS),\textsuperscript{25} isosorbide dinitrate (ISDN),\textsuperscript{26} and DETA/NO\textsuperscript{27}) or have antioxidant properties (tempol,\textsuperscript{5,6} apocynin,\textsuperscript{7,10} NAC,\textsuperscript{8} ramipril,\textsuperscript{9} folate,\textsuperscript{33} and aspirin). This indicates a NO redox imbalance in glucocorticoid-induced hypertension. It has been reported that aspirin stimulates NO production through acetylation of eNOS,\textsuperscript{31} and enhances its activity\textsuperscript{32} and increases intracellular cyclic GMP.\textsuperscript{32} Thus, the
potential mechanisms by which aspirin prevents and reverses ACTH-induced hypertension could be enhancing NO production and cyclic GMP availability, and decreasing superoxide-mediated NO degradation. The limitation of the present study is lack of reliable measurements for NO and superoxide levels in resistance vessels.

In the present study, both ACTH and Dex decreased body weight and thymus weight compared with saline-treated rats. These results are consistent with our previous findings.2–10 The effect of aspirin on body weight gain was inconsistent between groups. There was a mild decrease in daily water consumption in the initial days of aspirin-treated compared with non-aspirin-treated rats (data not shown). The ACTH but not Dex and saline increased daily water consumption. Body weight gain was lower in aspirin + saline- and aspirin + Dex-treated rats but higher in aspirin + ACTH-treated rats in prevention studies suggesting that the effect of aspirin on water consumption may contribute to its effect on body weight. Thymus weight was higher in the saline + aspirin group compared with saline alone treatment in a reversal study. However, there were no differences in the prevention study or in aspirin and glucocorticoid co-treatment groups and therefore this may be a chance finding. The fact that aspirin did not decrease thymus weight suggests its antihypertensive effect was not through an effect on glucocorticoid activity.

There are differences in the hypertension induced by ACTH and Dex in rats. L-Arginine prevents and partially reverses ACTH- but not Dex-induced hypertension.2–4,10 Dehydroepiandrosterone (DHEA), which is known to block Dex-induced hypertension, did not modify ACTH-induced hypertension in the rat.33 In the present study, the effects of aspirin on systolic BP in these two models are dissimilar in extent. These results suggest differences in mechanism between ACTH- and Dex-induced hypertension.

In conclusion, aspirin prevented and partially reversed ACTH-induced hypertension, and tended to prevent but failed to reverse Dex-induced hypertension in SD rats. Whether aspirin has a role in treatment of glucocorticoid-induced hypertension in humans remains to be determined.

References