Effect of Acetylsalicylic Acid and AT1 Receptor Antagonism on Vascular Oxidative Stress in Hypertensive Rats

AL-RIDI M.R.[1]*, ABDEL-SATER K.A.[1] and SOKAR S.S.[2]

[1] Department of Medical Physiology, Faculty of Medicine, King Abdulaziz University, Saudi Arabia
[2] Department of Pharmacology & Toxicology, Faculty of Pharmacy, Tanta University, Egypt
E-mail: melridi@yahoo.com

Abstract: This study investigated the effects of losartan (Los; AT1 receptor antagonist) and acetylsalicylic acid (Aspirin; Asp) on dexamethasone (Dex)-induced hypertensive rats. Fifty adult male Wistar rats were used and divided into 5 equal groups; Control group (***Con***); Hypertensive group (***HT0***) i.p. injected with Dex (40 μg/kg/d); Hypertensive Los-treated group (***HTL***) p.o. administered with Los (40 mg/kg/d); Hypertensive Asp-treated group (***HTA***) p.o. administered with Asp (100 mg/kg/d); and Hypertensive Los- & Asp-treated group (***HTLA***). Induced hypertension was associated with decreased thymus weight (ThW), increased aortic vascular reactivity (AVR) to norepinephrine (NE), attenuated relaxation to the endothelium-dependent vasodilator acetylcholine (ACh) but not to the endothelium-independent vasodilator sodium nitroprusside (SNP), decreased renal blood flow velocity (RBV) with increased renal blood flow resistance (RBR), decreased aortic tissue superoxide dismutase (SOD), glutathione peroxidase (GPX) & catalase (CAT), decreased plasma nitric oxide metabolites (NOx) & reduced glutathione (GSH), and increased plasma malondialdehyde (MDA). HTL & HTA rats showed significantly attenuated Dex-induced effects on all tested parameters, except ThW. HTLA rats showed complete preventive effects on systolic blood pressure (SBP), AVR to NE & ACh, RBV, RBR, and plasma MDA, NOx & GSH. Moreover, that group showed greater enhancement of aortic tissue GPX & CAT activity.
[AL-RIDI M.R., ABDEL-SATER K.A. and SOKAR S.S.. **Effect of Acetylsalicylic Acid and AT1 Receptor Antagonism on Vascular Oxidative Stress in Hypertensive Rats.** *Life Sci J* 2013; 10(2): 2638-2648]. (ISSN: 1097-8135). http://www.lifesciencesite.com 366

Key words: aspirin, losartan, dexamethasone, hypertension, oxidative stress, vascular endothelium, antioxidants

INTRODUCTION

Endothelial dysfunction is a characteristic of patients with essential hypertension (HT) (Miloradovic et al., 2008). By definition, endothelial dysfunction is a functional and reversible alteration of endothelial cells, resulting from oxidative stress and impairment of nitric oxide (NO) availability. Increased levels of biomarkers of oxidative stress and lipid peroxidation have been found in patients with HT (Nadar et al., 2004). Renal endothelial NO synthase (eNOS) expression is reduced in both adrenocorticotropic hormone-induced and corticosterone-induced hypertension in rats (Lou et al., 2001). In addition, glucocorticoid-induced HT is associated with decreased plasma reactive nitrogen intermediates (NOx) concentration (Wen et al., 2000) and enhanced markers of tissue superoxide production in rat aorta (Zhang et al., 2005).

Angiotensin II (Ang II) type 1 receptor (AT1) antagonists, including losartan (Los), are popular drugs frequently prescribed for treatment of HT or heart failure, and experimental data suggest that AT1 antagonists have the potential to improve endothelium-mediated vasomotion (Hornig et al., 2003). There is increasing experimental evidence that AT1 antagonists can enhance endothelium-dependent relaxation and that this effect is, in part, mediated by

eNOS and NADPH oxidase (Dal-Ros et al., 2010). So far, however, it is unclear whether AT1 antagonists affect the activity of vascular antioxidative enzyme systems. In addition, many patients with HT take antihypertensive drugs and aspirin (Asp) (Wu et al., 2004). It is widely used in clinical practice. It is an anti-inflammatory and cardiovascular protective drug. Aspirin has been proved to have potent inhibitor effects on cyclooxygenase (COX), and thus, COX-dependent generation of reactive oxygen species (ROS) (Taubert et al., 2004). The main benefits of Asp might appear to be anti-thrombotic, but recent interest has been directed to the anti-inflammatory and antioxidative properties of Asp. For example Asp protects the endothelium from the deleterious effects of ROS and enhanced endothelial production of NO both in vitro (Grosser & Schroder, 2003) and in vivo (Taubert et al., 2004). Moreover, Asp has been shown to prevent glucose-induced HT and superoxide overproduction in rats (El Midaoui et al., 2002), and reduce aortic and cardiac production of superoxide by lowering NADPH oxidase activity (Wu et al., 2004). However, only high dose of Asp (100 mg/kg/d) was shown to prevent Ang II-induced HT in rats (Wu et al., 2004). This dose was used in

the current study.

There is limited information about possible Asp interactions with AT1 antagonists, except for small studies (Fossum et al., 2005) and experimental data (Zhu et al., 2003). However, possible common pathways for Asp and Ang II type 2 receptors (AT2) were suggested, and the AT2, which are stimulated during AT1-blockade, may be involved in bradykinin (BK) production (de Gasparo & Siragy, 1999). Thus, a pharmacologic rationale for a possible AT1 antagonism and Asp interaction exists. Furthermore, because the exact signaling pathways and function of the other Ang II receptors are still in part unknown, interactions may be present even though the mode of action is unknown. Because of the common use of both Asp and AT1 antagonists and the lack of data about possible interactions from large-scale clinical trials, the present study was undertaken to (1) investigate the effects of Asp, a potent antioxidant, on Dex-induced hypertension in rats, (2) investigate and compare the effects of Asp or Los alone and in combination on vascular endothelial function, and (3) give a pharmacologic rationale for a possible AT1 antagonist/Asp interaction.

MATERIALS AND METHODS

Chemicals and Drugs: Dexamethasone (Dex; Fortcortin® ampoules; 8 mg/2 ml; Dex -21-dihydrogen phosphate) was purchased from Galaxo Smithkline (Cairo, Egypt). Losartan (Los; losartan® tablets; 100 mg/tablet) was obtained from Amriya Pharmaceutical Industries (Alexandria, Egypt). Acetylsalicylic acid (Aspirin; Asp; Aspocid® tablets; 250 mg/tablet) was obtained from Chemical Industries Development "CID" (Cairo, Egypt). Biochemical analysis was measured using assay kits purchased form Bio-diagnostic® (Cairo, Egypt). Normal saline (sodium chloride 0.9%) was purchased from Al-Gomhoria Pharmaceutical Co. (Cairo, Egypt). Norepinephrine (NE), acetylcholine (ACh), and sodium nitroprusside (SNP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Animals and Experimental Protocol: Fifty adult male Wistar rats [body weight (BW) = 221 ± 17 g; number (n) = 50] were used in this study. Rats were provided with standard rodent lab chow (El-Nasr Chemical Company, Cairo, Egypt) and tap water ad libitum. Animals were acclimatized for surroundings, handling, and equipment for systolic blood pressure (SBP) measurement for 1 week (wk) before experiments. Rats were equally divided into 5 groups (n = 10); (1) Normotensive non-treated control group (Con) injected intraperitoneally (i.p.) with saline (1 ml/kg/day) for 14 days (ds) and concomitantly orally (p.o.) administered with distilled water (1 ml/kg/d); (2) Hypertensive non-

treated group (HT0) injected i.p. with Dex (40 μg/kg/d; according to Miao et al., 2007) for 14 ds and concomitantly administered p.o. with distilled water as in group 1; (3) Hypertensive losartan-treated group (HTL) injected i.p. with Dex for 14 ds as in group 2 and concomitantly administered p.o. with the specific angiotensin-II type-1 (AT1) receptor antagonist, Los (40 mg/kg/d; according to Zhu et al., 2003); (4) Hypertensive aspirin-treated group (HTA) injected i.p. with Dex for 14 ds as in group 2 and concomitantly administered p.o. with Asp (100 mg/kg/d; according to Zhang et al., 2007); (5) Hypertensive combined losartan & aspirin-treated group (HTLA) injected i.p. with Dex for 14 ds as in group 2 and concomitantly administered p.o. with both Los and Asp as in groups 3 and 4 respectively.

During the experimental period, SBP and BW were measured on alternative days in all conscious rats. Animals were fasted for 12 h before the end of experiments, then they were anesthetized with sodium pentobarbital (50 mg/kg i.p.; according to Wu et al., 2005) and the left renal artery was exposed to measure renal blood flow velocity (RBV) & resistance (RBR). After that retro-orbital blood samples were collected from all rats where plasma samples were immediately separated and kept in freezer for further biochemical analysis. Rats were then sacrificed by cervical decapitation and thymus and aortic ring specimen were taken from each rat. Thymuses were weighed as markers of Dex activity (Zhang et al., 2007), and each aortic specimen was immediately divided into two pieces, one for measurements of tissue antioxidants, and the other one for aortic vascular reactivity (AVR) study.

All of the procedures regarding the care and use of animals and animal experimentation in this study were complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Measurement of SBP: SBP (in mmHg) was measured at 9-11 a.m. using a rat-tail sphygmomanometer (pneumatic transducer; Harvard apparatus Ltd, Aden Berge, U.K.) method and recorded on a physiograph (MK III-S; Narco Bio-System, U.S.A.). Rats were placed into restrainers on a heated plate (39°- 40°C) for 30 min. Several SBP measurements were recorded from each rat and the mean of 4 recordings (among which the difference was no more than 10 mmHg) was accepted as the SBP (Zhang et al., 2007).

Measurement of Renal Blood Flow Velocity and Resistance: Each anesthetized rat was laid on its back and mid-line laparotomy was made to expose the left renal artery. After setting the mode of pulsed blood flow meter (Doppler; Bi-Directional blood flow meter, HADECO, Smartdop 50, Japan),

ultrasonic gel was applied to the probe tip and the volume control was turned to maximum. The probe was pressed softly to the measured area at an angle of 45° - 50°. After hearing the optimal sounds and waiting for 5 sec without moving the probe, the freeze key was then pressed to freeze the waveform. Velocity (in cm/sec) and resistance (in PRU; peripheral resistance unit) were read from the printed strip paper (Haywood et al., 1981).

Biochemical Analysis: Plasma was separated from blood samples for determination of plasma malondialdehyde (MDA), reduced glutathione (GSH) and NO metabolites (NOx) nitrite and nitrate. One piece of each aortic ring specimen was homogenized in ice-cold KCl_4 (150 mM). The ratio of tissue weight to homogenization buffer was 1:10. From the latter, suitable dilutions were used to determine tissue levels of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT). MDA, a reactive aldehyde that is a measure of lipid peroxidation, was determined according to Uchiyama & Mihara (1979). The formed adducts following the reaction of plasma samples with thiobarbituric acid in boiling water bath, were extracted with n-butanol. The difference in optical density developed at two distinct wavelengths; 535 nm and 525 nm was a measure of the plasma MDA content expressed as nmol/L. GSH, an endogenous antioxidants, was determined according to Sedlak & Lindsay (1968). The procedure is based on the reduction of bis-(3-carboxy-4-nitrophenyl) disulfide reagent by SH group to form 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color that was measured spectrophotometrically at 412 nm. NOx determination was based on the method of Moshage et al. (1995) that depends on Griess reaction with a prior reduction step to convert nitrate to nitrite ions. Briefly, ethanol was added to the plasma in order to precipitate the proteins. Samples were centrifuged at 3000 rpm for 15 min. 0.5 ml of vanadium chloride was added rapidly to 0.5 ml of supernatant followed by addition of 0.5 ml of Griess reagent. The absorbances of samples were measured at 540 nm. GPX activity was determined in a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate (Paglia & Valentine, 1967). GPX activity was expressed in mU/g tissue. CAT activity was determined according to Aebi (1984). CAT reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with CAT inhibitor. The absorbance of samples was read at 510 nm against a standard blank. CAT activity was expressed in U/g tissue. SOD activity was determined according to Nishikimi et al. (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. SOD activity was expressed in U/g tissue.

Measurement of Aortic Vascular Reactivity: In each animal, a medial laparotomy was performed to excise the thoracic aorta. The vessel was cut into 6-mm ring and gently dissected free of fat and connective tissue. Rings were then cut into two pieces (3-mm each). One piece was mounted into 10-ml organ baths filled with carbogen (95% O_2/5% CO_2) that consisting of (in mmol/L) NaCl 118, KCl 4.75, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.03, and glucose 11; pH 7.4; temperature 37°C. The preparation was connected to a strain gauge (Grass, FT 03) isometric tension transducer that was connected to the pin chart recorder of the physiograph (MK III-S; Narco Bio-System, U.S.A.). The rings were subjected to an initial resting tension of 1 g and kept in the organ bath for equilibration for approximately 2 hs and washed every 15 min. After resting tension stabilization, NE (10-5 M) was administered to induce a rapid increase of vascular tone, which was followed by a stable vasoconstriction (Bornia et al., 2008). The aortic rings were then allowed to equilibrate for another 30 min before construction of cumulative concentration-effect curves to acetyl choline (ACh) and sodium nitroprusside (SNP) (Mondo et al., 2006). NE, ACh and SNP were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Body and Thymus Weights: The change in BW for each rat was calculated by subtracting the weight before treatment from the weight at d 14. Thymus wet weight (ThW) for each rat expressed as mg/100 g BW.

Statistical Analysis: All data were presented as means ± standard error of the means (SE). Comparison between groups was carried out by one way analysis of variance (ANOVA) followed by Dunnett t (2-sided) multiple comparisons to detect significant differences within individual means of all groups. The level of significance was set at $p \leq 0.05$ (Smoller, 2004). SBP, BW and vasorelaxation responses of aortic rings within and between groups were analyzed by repeated measures ANOVA. Statistical analysis was generated using SPSS software for windows, version 14.0, SPSS Inc., Chicago, IL, USA.

RESULTS

Body and Thymus Weights: The gain of BW by the end of 14 ds was significantly ($p < 0.001$) less in Dex-treated rats (HT0 group, 9.6 ± 1.1 g) compared with saline-treated rats (Con group, 54.4 ± 2.4 g). On the other hand, BW gain was significantly ($p < 0.001$) higher in hypertensive rats treated with

Los (HTL group), Asp (HTA group), or combined Los+Asp (HTLA group) than that of HT0 rats. Wet ThW was significantly (p < 0.001) less in HT0 rats (64.4 ± 2.3 mg/100 g BW) compared with Con rats (85.6 ± 1.9 mg/100 g BW). However, ThW did not change significantly (p > 0.05) by Los, Asp, or Los+Asp treatment when compared with that of HT0 value (Table 1).

Systolic Blood Pressure: Daily saline injection (Con group) did not significantly alter SBP (d0 111 ± 5.4, d14 107 ± 5.2 mmHg, p > 0.05), whereas Dex injection (HT0 group) significantly increased SBP (109 ± 4.8 to 180 ± 5.0 mmHg, p < 0.001). SBP was significantly increased in HTL (108 ± 6.3 to 141 ± 5.0 mmHg, p < 0.001) and HTA (106 ± 7.0 to 159 ± 6.7 mmHg, p < 0.001) rats, but insignificantly changed in HTLA (108 ± 6.0 to 112 ± 4.5 mmHg, p > 0.05) rats. At the end of study period (d14), SBP was significantly higher in Dex-treated rats than saline-treated rats (180 ± 5.0 vs. 107 ± 5.2 mmHg respectively, p < 0.001). Although hypertensive Los-treated (HTL) and Asp-treated (HTA) rats showed significantly lower (p < 0.001) SBP compared to HT0 rats, SBP in both groups was still significantly higher (p < 0.001) than that of Con rats. On the other hand, hypertensive combined Los+Asp-treated rats (HTLA) showed significantly lower (p < 0.001) SBP when compared to HT0, HTL & HTA rats, and insignificant difference (p = 0.732) when compared to Con group (Fig. 1).

Renal Blood Flow Velocity & Resistance: As shown in table (2), HT0 rats showed significantly (p < 0.001) lower RBV and significantly (p < 0.005) higher RBR compared with Con rats. Although RBV was significantly higher in HTL (p < 0.005) and HTA (p < 0.05) rats compared with HT0 animals, values were still significantly lower (p < 0.005, p < 0.001, respectively) than Con values. On the other hand, RBV in HTLA rats was significantly higher (p < 0.001) than that of HT0, but insignificantly different (p = 0.572) from that of Con group. In addition, RBR was significantly lower (p < 0.05) in HTL and HTA rats compared with HT0 rats, but only RBR of HTLA rats that was significantly lower (p < 0.05) than that of HT0 and insignificantly different (p = 0.799) from that of Con value.

Aortic Vascular Reactivity: Dex-treated (HT0) rats showed significantly higher (p < 0.001) AVR of aortic rings to NE compared with saline-treated (Con) rats (Fig. 2), and attenuated relaxation response to ACh (45.2 ± 6.1 % vs. 89.8 ± 5.1 %, p < 0.001) but not to SNP (85.3 ± 4.7 % vs. 92.5 ± 3.3 %, p = 0.614) as shown in Fig. (3). AVR to NE in HTL, HTA and HTLA rats were significantly lower (p < 0.001) than that of HT0 rats, but only HTLA rats showed AVR that was insignificantly (p = 0.206)

different form that of Con rats (Fig. 2). Moreover, HTL, HTA and HTLA rats showed significantly less attenuated relaxation responses to ACh (p < 0.05, p < 0.01, p < 0.005 respectively) when compared with that of the HT0 group, but only HTLA rats showed relaxation response to ACh that was insignificantly (p = 0.128) different from that of Con rats (Fig. 3).

Plasma NOx, MDA & GSH: Dex-treated (HT0) rats showed significantly lower (p < 0.001) plasma levels of NOx and GSH, and significantly higher (p < 0.001) MDA levels compared with Con rats (Table 3). Although HTL, HTA, and HTLA rats showed significantly higher levels of NOx (p < 0.005, p < 0.001, p < 0.001 respectively) and GSH (p < 0.001), and significantly lower (p < 0.001, p < 0.005, p < 0.001 respectively) MDA levels compared with HT0 rats, only HTLA rats showed insignificant differed of NOx, GSH and MDA levels (p = 0.130, p = 0.111, p = 0.765 respectively) when compared with the Con group (Table 3).

Aortic Tissue GPX, SOD & CAT: As shown in table (4), Dex-treated (HT0) rats showed significantly lower plasma levels of GPX, SOD and CAT (p < 0.001) compared with corresponding values of Con rats. Although HTL, HTA, and HTLA rats showed significantly (p < 0.005) higher levels of GPX, CAT and SOD when compared with HT0 rats, all of them were still significantly lower (p < 0.05) than normal values of Con (Table 4).

DISCUSSION

The present study confirmed previous findings that endothelial oxidative stress may play an important role in pathogenesis of HT. Dex injection in rats induced HT accompanied by increased AVR to NE with attenuated relaxation response to the endothelium-dependent vasorelaxant ACh (but not to the endothelium-independent vasorelaxant SNP). Moreover, RBV was decreased with increased resistance. These data suggests that the hypertensive effect of Dex is generalized and may be mediated through mechanisms that include both vascular endothelium and the kidneys. In agreement to the present results, Ang II-induced HT in rats was reported to be accompanied by decreased RBV and increased RBR (Sarkis et al., 2003). Moreover, incubation of rat aortic rings with captopril (ACE inhibitor) or Los attenuated the homocysteine-induced inhibition of endothelium-dependent relaxation caused by ACh (but not the endothelium-independent relaxation induced by SNP), and significantly resisted the decrease of NO content and elevation of MDA concentration in aortic tissues (Liu et al., 2007). In addition, the present study showed that Dex-induced HT was accompanied by NO redox imbalance that appeared as significant decrease in

plasma NOx, plasma GSH, aortic tissue antioxidants (SOD, GPX & CAT), and increase in the plasma index of lipid peroxidation, MDA. These data are in accordance with other results from human studies suggested that decrease in NO bioavailability and increases in oxidative stress are present in human HT (Touyz, 2004).

In the present study, rats co-treated with Dex and Los (HTL group) showed significantly attenuated Dex-induced HT, decreased RBV with increased resistance, and decreased AVR to NE and ACh (but not to SNP). Interestingly, HTL rats also showed improved oxidant/antioxidant status (increased plasma NOx and GSH, decreased plasma MDA, and increased aortic tissue antioxidants; GPX, CAT and SOD) compared with the HT0 group. These data suggest that the antihypertensive effect of Los is endothelium-dependent, and may be mediated via reduction of systemic oxidative stress mediators and enhancement of vascular endothelial antioxidants. This suggestion is supported by a recent study showed that treatment with Los increased NO production and reduced elevated SBP in spontaneously hypertensive rats (SHR) fed low-salt diet (Bayorh et al., 2007). In addition, it has been reported that plasma SOD, GPX, and NO levels were increased, while plasma MDA was reduced, in Los-treated rats compared with non-treated SHR. These findings denote that Los enhances the antioxidant capacity thereby improving the endothelial function (Zhu et al., 2007).

A possible mechanism related to the antihypertensive action of Los is via its interaction with NADPH oxidase. It was suggested that NADPH oxidase is a source of basal superoxide production in rat aortas. Oral administration of Los decreased the levels of superoxide and the expression and activity NADPH oxidase in the aortas of rats with adjuvant-induced arthritis (Sakuta et al., 2010). In addition, it has recently been reported that Ang II activates NADPH oxidase via its AT1 receptors leading to fibroblast growth and collagen synthesis in isolated mouse cardiac fibroblasts. These effects have been attenuated by Los and Asp (Wang et al., 2012). Moreover, aldosterone-salt-induced HT in rats was modestly prevented by Los. Aldosterone increased aortic NADPH oxidase activity by 34% and Los inhibited this activity. In addition, aortic expression of NADPH oxidase subunits increased in aldosterone-infused rats, which was decreased partially by Los (Park et al., 2008). Another possible mechanism of action of Los is via crosstalk between AT1 and AT2 receptors. AT1 and AT2 receptor are ideal candidates for maintaining a proper balance between the vasodilator agent NO and ROS. In pathological condition, stimulation of AT1 receptors

by increased Ang II levels produces oxidative stress response. In contrast, blockade of AT1 receptors, which is accompanied by increased Ang II, stimulates AT2 receptor and oppose the effect of AT1 receptor activity; a mechanism that appears to be involved in the beneficial effects of Ang II receptor blockers (Cipollone et al., 2004). This effect has recently been supported by finding that the combination of Los and AT2 agonist C21 enhanced the endothelium-dependent vascular relaxation in response to ACh in spontaneously hypertensive rats (Rehman et al., 2011). A third possible mechanism of action of Los is via interaction with bradykinin (BK) receptors. It was observed that AT1 antagonists enhanced flow-dependent endothelium-dependent relaxation in coronary artery disease; an effect that was inhibited by either icatibant (a BK receptor antagonist) and/or L-NMMA, suggesting that both BK and NO contribute to the vascular effects of AT1-receptor antagonists (Hornig et al., 2003).

The present study demonstrated that rats co-treated with Dex and Asp (HTA group) showed significantly improved antioxidant status (increased plasma NOx and GSH, decreased plasma MDA, and increased aortic tissue antioxidants GPX, CAT, SOD) compared with the HT0 rats. In addition, they also showed significantly attenuated Dex-induced HT, AVR to NE and ACh (but not to SNP). These data suggest that Asp in the given dose does not only improve Dex-induced endothelial dysfunction by attenuating the induced oxidative stress, but also has a significant endothelium-dependent antihypertensive action. These results are consistent with the antihypertensive effect of Asp demonstrated previously in other rat HT models. For example, chronic treatment of fructose-fed rats with aspirin has been shown to partially reverse the increment in SBP. In addition, Asp treatment (100 mg/kg) prevented the rise in SBP in hypercholesterolemic rats and significantly decreased lipid peroxidation (indicated by decreased serum MDA levels) and significantly increased serum GSH content (Tauseef et al., 2007). In addition, Asp dose-dependently reduced aortic tissue/smooth muscle cell superoxide production and NADPH oxidase activity in both normotensive and Ang II-induced hypertensive rats. High dose Asp (100 mg/kg/d) but not low dose (10 mg/kg/d) prevented glucocorticoid- or Ang II-induced HT and superoxide over production (Wu et al., 2004).

The antihypertensive effect of Asp can be attributed to several mechanisms of action. For example, Ang II-induced HT and overproduction of superoxide, was associated with selectively increased cardiac COX expression; an effect that was totally prevented by Asp treatment. Asp significantly attenuated Ang II-induced oxidative stress, HT, and

cardiac NADPH oxidase expression. Therefore, the antihypertensive effect of Asp was presumably acting through its antioxidative properties, in particular, inhibition of NADPH oxidase-mediated peroxide production (Wu et al., 2005). Another possible mechanism of Asp/Dex interaction is involvement of salt-induced superoxide production and increased NO bioavailability. Cortisol-induced HT is characterized by sodium retention and volume expansion. Salt loading was shown to significantly increase superoxide production and decrease NOS activity in basilar arteries of stroke-prone SHR; two effects which were significantly reversed by treatment with low-dose Asp (5 mg/kg/d for 5 wks). Thus, it has been suggested that low-dose Asp may exert protective effects against cerebrovascular inflammation and damage by salt loading through down-regulation of superoxide production and induction of NO synthesis (Ishizuka et al., 2008). In addition Asp effect may involves decrease of the atherogenic state. It has been reported that Asp-supplement to normal rat chow decreased serum total cholesterol, triglycerides, low-density lipoprotein (LDL) and VLDL with concomitantly increased HDL. It also decreased pancreatic and adipose lipid peroxidation (Sethi et al., 2011).

In the present study, there was a statistical positive interaction between treatment with Asp and Los in combination and Los or Asp alone, with complete prevention of Dex-induced HT, attenuated AVR to NE & ACh, decreased RBV, increased RBR, and altered plasma oxidant/antioxidant status (i.e. normalization of plasma levels of MDA, NO and GSH). Moreover, HTLA rats showed greater enhancement of aortic tissue GPX and CAT activity compared with Los- or Asp-treated rats (Table 4). These results could be attributed to the antioxidative properties of both drugs. Indeed, there is evidence that Los-based antihypertensive therapy combined with Asp was more effective than atenolol-based treatment combined with Asp, in reducing cardiovascular morbidity and mortality, and myocardial infarction in a large subset of the Losartan Intervention For Endpoint (LIFE) reduction in HT study participants. There was a statistical interaction between Los-treatment and aspirin in the LIFE study, with significantly greater reductions for myocardial infarction with Los in patients using Asp than in patients not using Asp at baseline (Fossum et al., 2005). Moreover, it has recently shown that there is a positive interaction between Los treatment and NO donors; among which is Asp. For example, in a recent study, adult male SHR were treated with Los (10 mg/kg) and with the NO donor L-arginine (2 g/kg) for 4 weeks, each drug alone and in combination. It has been reported that mean arterial BP was

significantly reduced in the Los group compared with the control group. Aortic blood flow was significantly higher and aortic vascular resistance was significantly lower in all treated groups than in the control. SOD activity rose significantly in the L-arginine plus Los group compared with control. The reported results suggested that combined treatment with Los and L-arginine supplementation has a beneficial effect on renal function that is, at least in part, mediated by increased SOD activity in SHR (Miloradovic et al., 2008).

In conclusion, the present study showed that HT is associated with vascular inflammatory and oxidative stress status, and the antihypertensive action of Los is endothelium-dependent, and may be mediated via reduction of systemic oxidative stress mediators and enhancement of vascular endothelial antioxidants. It also demonstrated that Asp (in the given dose) via its antioxidative properties, can improve Dex-induced vascular endothelial dysfunction, thus causing significant endothelium-dependent antihypertensive effect. Moreover, the study also reported a positive interaction between Los and Asp in treatment of Dex-induced HT in rats. However, many effects of Asp still continue to be discovered, and its effects on the vascular endothelium appear to be important. The later may be one of the mechanisms by which Asp can help in prevention of cardiovascular diseases among which is hypertension. It remains to be determined whether Asp has a role in treatment of glucocorticoid-induced HT in human. Nevertheless, specifically designed clinical trials are currently needed to document the effective pathogenic role of endothelial dysfunction in hypertension, and the possibility that its reversal, using antioxidants supplements, can add effective advantages in antihypertensive treatment.

*Correspondence author:
Dr. Al-Ridi, Mamdouh Ramadan,
Department of Medical Physiology, Faculty of Medicine, King Abdulaziz University, Saudi Arabia.
Cellular Phone: +966 56 839 7055
E-mail: melridi@yahoo.com

REFERENCES
[1] Miloradovic Z, Jovovicg D, Mihailovic-Stanojevic N, Milanovic JG and Milanovic S (2008) Effects of long-term losartan and L-

arginine treatment on haemodynamics, glomerular filtration, and SOD activity in spontaneously hypertensive rats. Can J Physiol Pharmacol. 86(4): 210-214.

[2] Nadar S, Blann AD and Lip GY (2004) Endothelial dysfunction: methods of assessment and application to hypertension. Curr Pharm Des. 10: 3591-3605.

[3] Lou YK, Wen C, Li M, Adams DJ, Wang MX, Yang F, Morris BJ and Whitworth JA (2001) Decreased renal expression of nitric oxide synthase isoforms in adrenocorticotropin-induced and corticosterone-induced hypertension. Hypertension. 37: 1164-1170.

[4] Wen C, Li M, Fraser T, Wang J, Turner SW and Whitworth JA (2000) L-Arginine partially reverses established adrenocorticotropin-induced hypertension and nitric oxide deficiency in the rat. Blood Press. 9: 298-304.

[5] Zhang Y, Chan MM, Andrews MC, Mori TA, Croft KD, MeKenzie KU, Schyvens CG and Whitworth JA (2005) Apocynin but not allopurinol prevents and reverses ACTH-induced hypertension in the rat. Am J Hypertens. 18: 910-916.

[6] Hornig B, Kohler C, Schlink D, Tatge H and Drexler H (2003) AT1-receptor antagonism improves endothelial function in coronary artery disease by bradykinin /B2-receptor-dependent mechanism. Hypertension. 41: 1092-1095.

[7] Dal-Ros S, Oswald-Mammosser M, Pestrikova T, Schott C, Boehm N, Bronner C, Chataigneau T, Geny B and Schini-Kerth VB (2010) Losartan prevents portal hypertension-induced, redox-mediated endothelial dysfunction in the mesenteric artery in rats. Gastroenterology. 138(4): 1574-1584.

[8] Wu R, Laplante MA and De Champlain J (2004) Prevention of angiotensin II-induced hypertension, cardiovascular hypertrophy and oxidative stress by acetylsalicylic acid in rats. J Hypertens. 22(4): 793-801.

[9] Taubert D, Berkels R, Grosser N, Schroder H, Grundemann D and Schomig E (2004) Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol. 143(1): 159-165.

[10] Grosser N and Schroder H (2003) Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. Arterioscler Thromb Vasc Biol. 23(8): 1345-1351.

[11] El Midaoui A, Wu R and de Champlain J (2002) Prevention of hypertension, hyperglycemia and vascular oxidative stress by aspirin treatment in chronically glucose-fed rats. J Hypertens. 20(7): 1407-1412.

[12] Fossum E, Moan A, Kjeldsen SE, Devereux RB, Julius S, Snapinn SM, Edelman JM, de Faire U, Fyhrquist F, Ibsen H, Kristianson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H and Dahlof B (2005) The effect of losartan versus atenolol on cardiovascular morbidity and mortality in patients with hypertension taking aspirin: the Losartan Intervention for Endpoint Reduction in hypertension (LIFE) study. J Am Coll Cardiol. 46(5): 770-775.

[13] Zhu BQ, Sievers RE, Browne AE, Lee RJ, Chatterjee K, Grossman W, Karliner JS and Parmley WW (2003) Comparative effects of aspirin with ACE inhibitor or angiotensin receptor blocker on myocardial infarction and vascular function. J Renin Angiotensin Aldosterone Syst. 4(1): 31-37.

[14] de Gasparo M and Siragy HM (1999) The AT2 receptor: fact, fancy and fantasy. Regul Peptides. 81: 11–24.

[15] Miao Y, Zhang Y, Lim PS, Kanjanapan Y, Mori TA, Croft KD, Earl J, Lee SY, McKenzie KU, Hu L and Whitworth JA (2007) Folic acid prevents and partially reverses glucocorticoid-induced hypertension in the rat. Am J Hypertens. 20(3): 304-310.

[16] Zhang Y, Miao Y and Whitworth JA (2007) Aspirin prevents and partially reverses adrenocorticotropic hormone-induced hypertension in the rat. Am J Hypertens. 20(11): 1222-1228.

[17] Wu R, Laplante MA and de Champlain J (2005) Cyclooxygenase-2 inhibitors attenuate angiotensin II-induced oxidative stress, hypertension, and cardiac hypertrophy in rats. Hypertension. 45(6): 1139-1144.

[18] Haywood JR, Shaffer RA, Fastenow C, Fink GD and Brody MJ (1981) Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat, Am J Physiol Heart Circ Physiol. 241: H273-H278.

[19] Uchiyama M and Mihara M (1979) Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 86: 271–278.

[20] Sedlak J and Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 25(1): 192-205.

[21] Moshage H, Kok B, Huizenga JR and Jansen PL (1955) Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem. 41(6 Pt 1): 892-896.

[22] Paglia DE and Valentine WN (1967) Studies on the quantitative and qualitative characterization

of erythrocyte glutathione peroxidase. J Lab Clin Med. 70(1): 158-169.

[23] Aebi H. Catalase in vitro (1984) Methods Enzymol. 105: 121-126.

[24] Nishikimi M, Appaji N and Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 46(2): 849-854.

[25] Bornia EC, do Amaral V, Bazotte RB and Alves-Do-Prado W (2008) The reduction of arterial tension produced by stevioside is dependent on nitric oxide synthase activity when the endothelium is intact. J Smooth Muscle Res. 44(1): 1-8.

[26] Mondo CK, Yang WS, Zhang N and Huang TG. Anti-oxidant effects of atorvastatin in dexamethasone-induced hypertension in the rat (2006) Clin Exp Pharmacol Physiol. 33(11): 1029-1034.

[27] Smoller SW (2004) Biostatistics and Epidemiology: A primer for health and biomedical professionals, 3rd edition. Springer-Verlag Inc., New York, USA. pp: 69-77.

[28] Sarkis A, Liu KL, Lo M and Benzoni D (2003) Angiotensin II and renal medullary blood flow in Lyon rats. Am J Physiol Renal Physiol. 284(2): F365-F372.

[29] Liu YH, You Y, Song T, Wu SJ and Liu LY (2007) Impairment of endothelium-dependent relaxation of rat aortas by homocysteine thiolactone and attenuation by captopril. J Cardiovasc Pharmacol. 50(2): 155-161.

[30] Touyz RM (2004) Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension. 44(3): 248-252.

[31] Bayorh MA, Layas MF, Mann G, Feuerstein GZ and Eatman D (2007) The effect of diet on simvastatin and losartan enhancement of endothelial function. Clin Exp Hypertens. 29(5): 311-325.

[32] Zhu WW, Liu XP, Wu N, Zhao TT, Zhao Y, Zhang J and Shao JH (2007) Beneficial effects of losartan on vascular injury induced by advanced glycosylation end products and their receptors in spontaneous hypertension rats. Mol Cell Biochem. 304(1-2): 35-43.

[33] Sakuta T, Morita Y, Satoh M, Fox DA and Kashihara N (2010) Involvement of the renin-angiotensin system in the development of vascular damage in a rat model of arthritis: effect of angiotensin receptor blockers. Arthritis Rheum. 62(5): 1319-1328.

[34] Wang X, Lu J, Khaidakov M, Mitra S, Ding Z, Raina S, Goyal T and Mehta JL (2012) Aspirin suppresses cardiac fibroblast proliferation and collagen formation through downregulation of angiotensin type 1 receptor transcription. Toxicol Appl Pharmacol. 259(3):346-354.

[35] Park YM, Lim BH, Touyz RM and Park JB (2008) Expression of NAD(P)H oxidase subunits and their contribution to cardiovascular damage in aldosterone/salt-induced hypertensive rat. J Korean Med Sci. 23(6): 1039-1045.

[36] Cipollone F, Fazia M, Iezzi A, Pini B, Cuccurullo C, Zucchelli M, de Cesare D, Ucchino S, Spigonardo F, De Luca M, Muraro R, Bei R, Bucci M, Cuccurullo F and Mezzetti A (2004) Blockade of the angiotensin II type 1 receptor stabilizes atherosclerotic plaques in humans by inhibiting prostaglandin E2-dependent matrix metalloproteinase activity. Circulation. 109(12): 1482-1488.

[37] Rehman A, Leibowitz A, Yamamoto N, Rautureau Y, Paradis P and Schiffrin EL (2011) Angiotensin type 2 receptor agonist compound 21 reduces vascular injury and myocardial fibrosis in stroke-prone spontaneously hypertensive rats. Hypertension. 59(2): 291-299.

[38] Tauseef M, Sharma KK and Fahim M (2007) Aspirin restores normal baroreflex function in hypercholesterolemic rats by its antioxidative action. Eur J Pharmacol. 556(1-3): 136-143.

[39] Ishizuka T, Niwa A, Tabuchi M, Ooshima K and Higashino H (2008) Acetylsalicylic acid provides cerebrovascular protection from oxidant damage in salt-loaded stroke-prone rats. Life Sci. 82(13-14): 806-815.

[40] Sethi A, Parmar HS and Kumar A (2011) The effect of aspirin on atherogenic diet-induced diabetes mellitus. Basic Clin Pharmacol Toxicol. 108(6): 371-377.

6/20/2013

Table 1. Effect of aspirin and/or losartan treatment on changes in body weight (BW) and thymus weight (ThW) measured in dexamethasone-hypertensive groups of rats.

Parameters	Groups of rats				
	Con	HT0	HTL	HTA	HTLA
Change in BW (g)	54.4 ±2.4	9.6 ±1.1 *	42.6 ±1.5 *#	45.2 ±1.5 *#	52.8 ±2.8 #
ThW (mg/100 g BW)	85.6 ±1.9	64.4 ±2.3 *	68.4 ±2.7 *	67.2 ±1.7 *	69.4 ±3.6 *

Con = normotensive non-treated control group; HT0 = hypertensive non-treated group; HTL = hypertensive losartan-treated group; HTA = hypertensive aspirin-treated group; HTLA = hypertensive combined losartan & aspirin-treated group.
Values are presented as means ± S.E. (Number of rats in each group is 10). * = Significant difference of the specified group from Con group (P ≤ 0.05). # = Significant difference of the specified treated group from HT0 group (P ≤ 0.05).

Table 2. Effect of aspirin and/or losartan treatment on renal blood flow velocity (RBV) and resistance (RBR) measured in dexamethasone-hypertensive groups of rats.

Parameters	Groups of rats				
	Con	HT0	HTL	HTA	HTLA
RBV (cm/sec)	7.8 ±0.5	2.3 ±0.2 *	4.0 ±0.5 *#	3.8 ±0.3 *#	7.2 ±0.7 #
RBR (PRU)	0.8 ±0.04	1.2 ±0.02 *	0.9 ±0.05 *#	1.0 ±0.07 *#	0.8 ±0.03 #

Con = normotensive non-treated control group; HT0 = hypertensive non-treated group; HTL = hypertensive losartan-treated group; HTA = hypertensive aspirin-treated group; HTLA = hypertensive combined losartan & aspirin-treated group; PRU = peripheral resistance unit.
Values are presented as means ± S.E. (Number of rats in each group is 10). * = Significant difference of the specified group from Con group (P ≤ 0.05). # = Significant difference of the specified treated group from HT0 group (P ≤ 0.05).

Table 3. Effect of aspirin and/or losartan treatment on plasma levels of NO metabolites (NOx), lipid peroxidation maker (malondialdehyde, MDA), and antioxidant reduced glutathione (GSH) measured in all groups of rats.

Parameters	Groups of rats				
	Con	HT0	HTL	HTA	HTLA
NOx (mmol/l)	13.3 ±0.7	4.8 ±0.4 *	7.3 ±0.5 *#	9.1 ±0.7 *#	15.8 ±1.2 #
MDA (mmol/l)	8.3 ±0.4	15.6 ±0.9 *	11.4 ±0.8 *#	11.0 ±0.7 *#	8.0 ±0.6 #
GSH (mmol/l)	10.3 ±0.7	2.9 ±0.3 *	4.9 ±0.4 *#	6.1 ±0.7 *#	9.6 ±0.6 #

Con = normotensive non-treated control group; HT0 = hypertensive non-treated group; HTL = hypertensive losartan-treated group; HTA = hypertensive aspirin-treated group; HTLA = hypertensive combined losartan & aspirin-treated group.
Values are presented as means ± S.E. (Number of rats in each group is 10). * = Significant difference of the specified group from Con group (P ≤ 0.05). # = Significant difference of the specified treated group from HT0 group (P ≤ 0.05).

Table 4. Effect of aspirin and/or losartan treatment on aortic tissue antioxidants (glutathione peroxidase, GPX; superoxide dismutase, SOD; catalase, CAT) measured in all groups of rats.

Parameters	Groups of rats				
	Con	HT0	HTL	HTA	HTLA
GPX (mU/g tissue)	44.9 ±2.8	7.9 ±0.9 *	26.0 ±2.7 *#	31.8 ±2.2 *#	38.6 ±2.1 *#
SOD (U/g tissue)	451 ±16.7	311 ±11.1 *	372 ±14.7 *#	391 ±11.6 *#	411 ±18.2 *#
CAT (U/g tissue)	9.6 ±0.2	4.1 ±0.2 *	6.6 ±0.4 *#	6.8 ±0.3 *#	8.8 ±0.3 *#

Con = normotensive non-treated control group; HT0 = hypertensive non-treated group; HTL = hypertensive losartan-treated group; HTA = hypertensive aspirin-treated group; HTLA = hypertensive combined losartan & aspirin-treated group.
Values are presented as means ± S.E. (Number of rats in each group is 10). * = Significant difference of the specified group from Con group (P ≤ 0.05). # = Significant difference of the specified treated group from HT0 group (P ≤ 0.05).
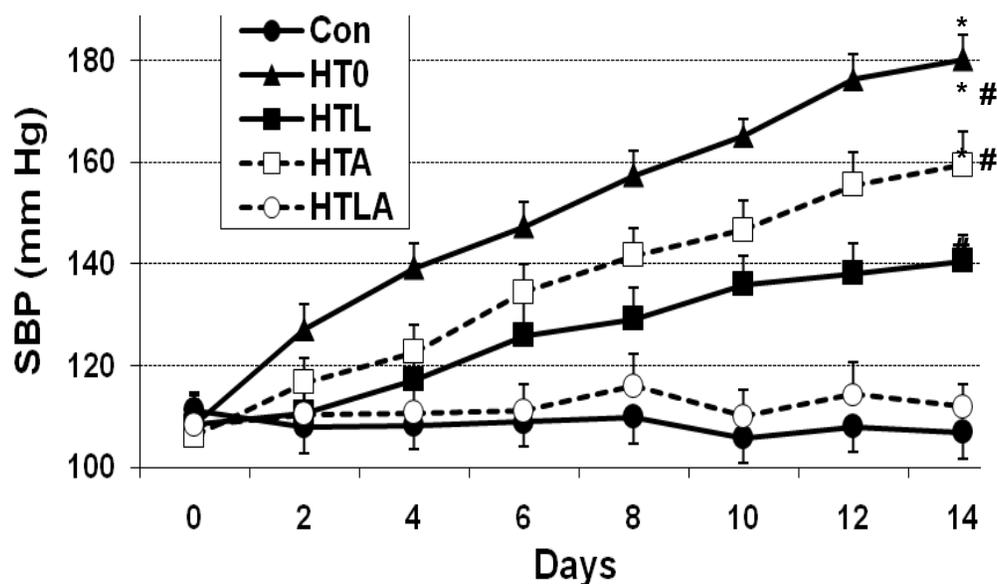
Figure 1. Systolic blood pressure (SBP) in normotensive non-treated (Con), hypertensive non-treated (HT0), hypertensive losartan-treated (HTL), hypertensive aspirin-treated (HTA) and hypertensive combined losartan & aspirin-treated (HTLA) rats.

Data are shown as means $\pm$ S.E. (Number of rats in each group is 10). * = Significant difference of the specified group from Con group (P $\leq$ 0.05). # = Significant difference of the specified treated group from HT0 group (P $\leq$ 0.05).
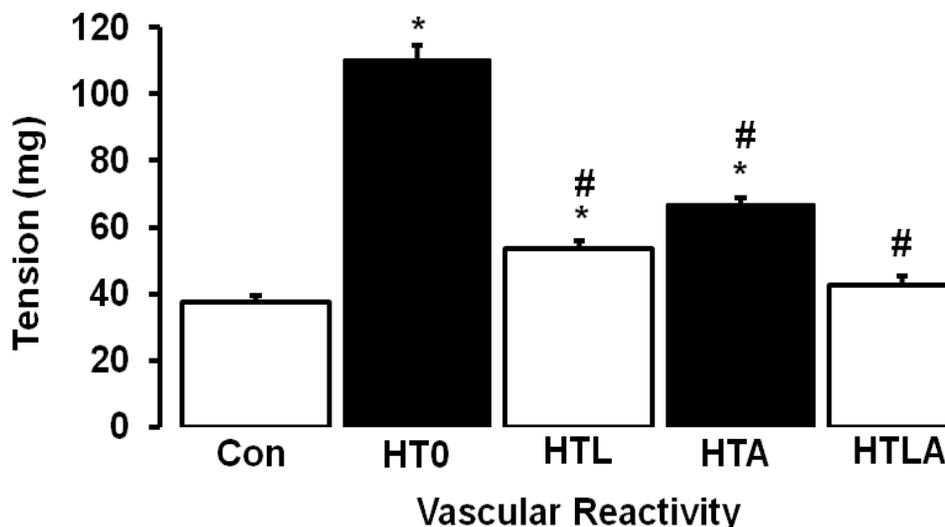

Figure 2. Vascular reactivity of aortic rings (mg tension) to 1×10^{-5} M norepinephrine in normotensive non-treated (Con), hypertensive non-treated (HT0), hypertensive losartan-treated (HTL), hypertensive aspirin-treated (HTA) and hypertensive combined losartan & aspirin-treated (HTLA) rats.

Data are shown as means $\pm$ S.E. (Number of rats in each group is 10).
* = Significant difference of the specified group from Con group (P $\leq$ 0.05).
# = Significant difference of the specified treated group from HT0 group (P $\leq$ 0.05).
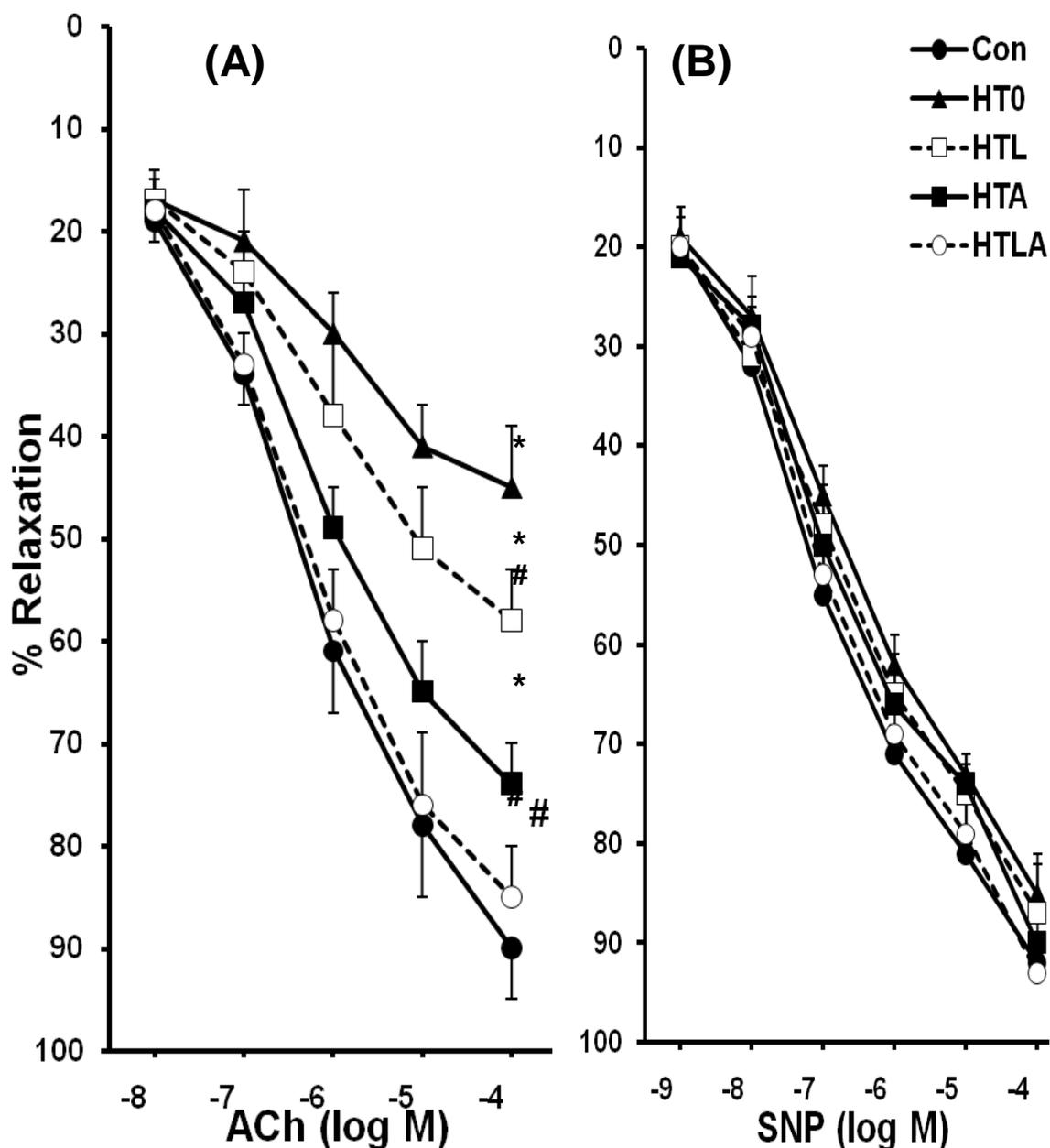
Figure 3. Vasorelaxation of aortic rings in response to **(A)** endothelium-dependent acetylcholine (ACh), and **(B)** endothelium-independent sodium nitroprusside (SNP) measured in control (Con), hypertensive non-treated (HT0), hypertensive losartan-treated (HTL), hypertensive aspirin-treated (HTA) and hypertensive combined losartan & aspirin-treated (HTLA) rats.

Data are shown as means $\pm$ S.E. (Number of rats in each group is 10).

* = Significant difference of the specified group from Con group (P ≤ 0.05).

= Significant difference of the specified treated group from HT0 group (P ≤ 0.05).