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Effect of combination of renin inhibitor and Mas-receptor agonist in DOCA–salt-induced hypertension in rats

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Abstract To investigate the combined effect of aliskiren, a renin inhibitor, and AVE 0991, a Mas-receptor agonist, in experimental hypertension (HT) in rats. HT was produced by administration of deoxycorticosterone acetate (DOCA) and mean arterial blood pressure (MABP) was assessed by tail-cuff method. Treatments were started from 4th week onwards and were continued for 9 days. A significant increase in MABP was noted after 1 week in DOCA control rats, as compared with the base line value. A stable HT developed after 4 weeks of DOCA administration. Treatments with aliskiren and AVE 0991 alone, dose-dependently decreased MABP in DOCA-treated rats. Further, combination of low doses of aliskiren and AVE 0991 significantly reduced MABP, as compared with DOCA control rats and with either drug alone in low doses. It may be concluded that treatment with aliskiren produced down-regulation of both harmful Ang II–AT1-receptor and survival Ang(1–7)/Mas-receptor axis of RAAS. Treatment with combination of low doses of aliskiren and AVE 0991, for the first time, has been shown to produce synergistic blood pressure lowering effect. Therefore, combination of renin inhibitor with Mas-receptor agonist may prove beneficial for the treatment of hypertensive patients.

Keywords Aliskiren · AVE 0991 · DOCA–salt hypertension · Mas-receptor

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Introduction

Hypertension (HT) is an independent risk factor for various cardiovascular disorders such as arterial aneurysm, strokes, heart failure, and its prevalence is constantly increasing worldwide according to National Health and Nutrition Examination Survey data [1, 2]. The renin–angiotensin–aldosterone system (RAAS) is a very important regulatory system in cardiovascular and blood pressure homeostasis [3], and sustained over-activation of RAAS has been reported to produce end-organ damage in hypertensive patients.

Several preclinical and clinical studies have demonstrated that treatment with either angiotensin-converting enzyme inhibitors (ACEIs) [4] or ANG II type 1-receptor blockers (ARBs) [5] produce significant antihypertensive effect and provide end-organ protection. Furthermore, the beneficial effects of ACEIs and ARBs have been shown to be partly mediated through the activation of Ang1–7/Mas-receptor axis [6–8].

Angiotensin-converting enzyme-2 (ACE-2) is a zinc metallopeptidase which hydrolyses Ang I and II into Ang(1–9) and Ang(1–7) fragments, respectively; Ang(1–9) in turn is converted into Ang(1–7) by the enzymes ACE and endopeptidases [9, 10]. The Ang(1–7) produces vasodilation, antiproliferative, natriuretic activity through activation of Mas-receptor [11, 12]. Further, chronic treatment with angiotensin (1–7) significantly decreases dyslipidemia, a major culprit of cardio-renal dysfunctions in diabetic rats [13, 14]. Moreover, AVE 0991, a nonpeptide Mas-receptor agonist efficiently mimics the effects of angiotensin-(1–7) on the endothelium [15–17].

Renin is the rate-limiting step in the generation of Ang II. Recently, the nonpeptide renin inhibitor, aliskiren (ALK), was approved for the treatment of HT [18]. Animal

and clinical studies have revealed striking vasodilator effect of ALK [19]. Furthermore, ALK has proven to be beneficial in several other pathophysiological settings such as cardiac remodeling and fibrosis as well as atherosclerosis [20].

The hypothesis investigated in this study was that whether or not a complete blockade of RAAS with renin inhibitor would down-regulate the beneficial Ang(1–7)/Mas-receptor axis, and if it is so, whether supplementation with AVE-0991, a nonpeptide Mas-receptor agonist would produce an additive or synergistic effect in DOCA-induced HT in rats.

Materials and methods

Age matched Wistar rats of either sex weighing 180–240 g were employed in the present study. The care and the use of these animals were in accordance with the guidelines of the CPCSEA (committee for the purpose of control and supervision of experiments on animals). Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) (Protocol No. ISF/CPCSEA/IAEC/2010/16).

Deoxycorticosterone acetate-induced (DOCA–salt) experimental HT

DOCA-treated rats were developed HT as previously reported [21]. In brief, a midline abdominal incision was made under anesthesia with a ketamine solution and the left kidney was removed. After 15 days of surgery, administration of DOCA was initiated, twice weekly by subcutaneous injection in a suspension of 0.2 ml of olive oil and drinking water was replaced with 1 % NaCl and 0.2 % KCl [22]. Stable HT develops after 4 week of DOCA–salt administration. The animals were restrained and conditioned for 5 days before commencing blood pressure measurements. The rat tail was heated with 200 W bulb heat lamp for 3–5 min and mean arterial blood pressure (MABP) was recorded using noninvasive blood pressure measurement technique (NIBP-Biopac-MP 100; Goleta, CA, USA). At least three separate indirect pressures were averaged for each animal. The animals having MABP $\geq$180 mmHg were considered as a hypertensive [23].

Experimental protocol

This study consists of seven groups and each group comprises of 5–6 rats of either sex. ALK and AVE 0991 were dissolved in normal saline solution (0.9 %).

Group I (Sham control): rats were underwent sham surgery and were maintained on normal drinking water for whole study period.

Group II (DOCA control): uninephrectomized rats were administered DOCA (20 mg/kg/s.c., twice weekly, 4 weeks) and on 5–6 weeks rats were maintained on normal drinking water without being treated with DOCA.

Group III (high dose ALK-treated hypertensive rats): rats were treated with high dose of ALK (50 mg/kg/i.p.; 9 days) [24], 4-week following DOCA administration.

Group IV [low dose (sub-effective dose) ALK-treated hypertensive rats]: rats were treated with low dose ALK (25 mg/kg/i.p.; 9 days), 4-week following DOCA administration.

Group V (high dose AVE 0991-treated hypertensive rats): rats were treated with high dose of AVE 0991 (576 μg/kg/i.p.; 9 days) [25], 4-week following DOCA administration.

Group VI (low dose AVE-0991-treated hypertensive rats): rats were treated with low dose of AVE 0991(288 μg/kg/i.p.; 9 days), 4-week following DOCA administration.

Group VII (low dose ALK + low dose AVE 0991-treated hypertensive rats): rats were treated with low dose ALK (25 mg/kg/i.p.; 9 days) 60 min before treatment with low dose AVE-0991 (288 μg/kg/i.p.; 9 days), 4-week following DOCA administration.

Drugs and chemicals

DOCA–salt was obtained from Sigma, St. Louis, MO, USA. AVE 0991 was obtained from Sanofi-Aventis, Germany as an ex-gratia. ALK was obtained from Novartis; USA as an ex-gratia. All other chemicals used were of analytical grade.

Statistical analysis

Values were expressed as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test was used to determine the statistical significance ($P < 0.05$) between various groups.

Results

In group first, five animals were underwent sham surgery and were maintained on normal drinking water. There was no significant change observed in MABP during whole study period in these animals.

Effect of DOCA–salt on mean arterial blood pressure (MABP) in normal rats

A significant increase in MABP was noted after 1 week in DOCA-treated uninephrectomized rats, as compared with the base line value. A stable hypertension (201 ± 10 mmHg) developed after 4 week of DOCA administration. The MABP

remained significantly high even in the DOCA free period from 5th to 6th weeks (Fig. 1).

Effect of low and high dose ALK on MABP in DOCA-treated rats

A significant increase in MABP (185.6 ± 11.4 mmHg) and (195.6 ± 13.9 mmHg) was noted in DOCA-treated rats in both groups (low dose ALK and high dose ALK-treated groups), respectively, as compared with their baseline values. Treatment with the low dose ALK significantly decreased MABP starting from day 4 and a maximum decrease in MABP (49 ± 2.6 mm/Hg) was on 9th day as compared with DOCA-treated rats, but did not reach the basal value even at the end of experiment. Treatment with the high dose of ALK significantly decreased MABP from day 3 onward and reached to the basal value on 7th day of treatment (Figs. 2, 3; Table 1).

Effect of low dose and high dose AVE 0991 on MABP in DOCA-treated rats

A significant increase in MABP (187.3 ± 15.0 mmHg) and (190.3 ± 13.5 mmHg) was noted in DOCA–salt-treated rats in both groups (low dose AVE0991 and high dose AVE0991-treated groups), respectively, as compared with the baseline value. Treatment with the low dose AVE0991 significantly decreased MABP starting from day 4 onward and a maximum decrease in MABP (45 ± 3.2 mm/Hg) was on 9th day as compared with DOCA–salt-treated rats, but did not reach the basal value even at the end of experiment. Whereas treatment with the high dose of AVE0991 significantly decreased MABP starting from day 3 onwards and reached to the basal value on 6th day of treatment (Figs. 4, 5; Table 1).

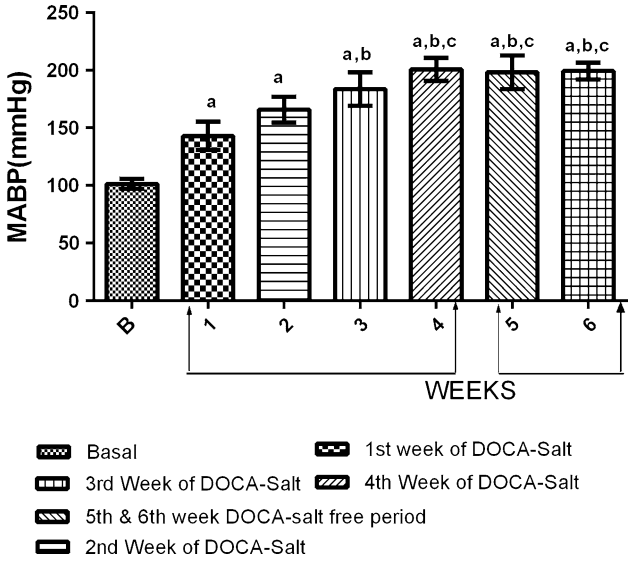

Fig. 1 Effect of DOCA–salt on MABP in normal rats: all values are expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus 1st week of DOCA–salt; *c* $P < 0.05$ versus 2nd week of DOCA–salt. *B* basal value ($n = 6$)

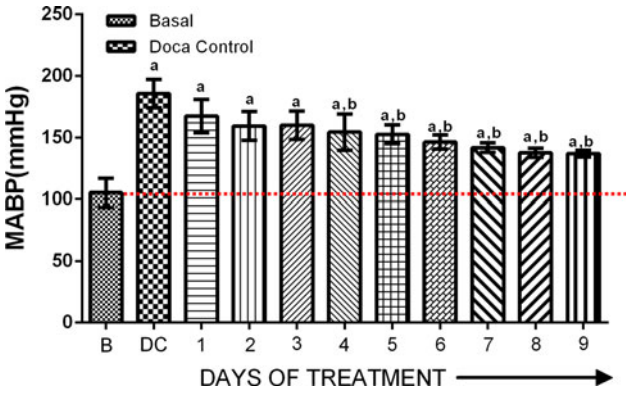

Fig. 2 Effect of low dose ALK on MABP in DOCA-treated rats: all values are expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus DOCA control. *B* basal value, *DC* DOCA control ($n = 5$)

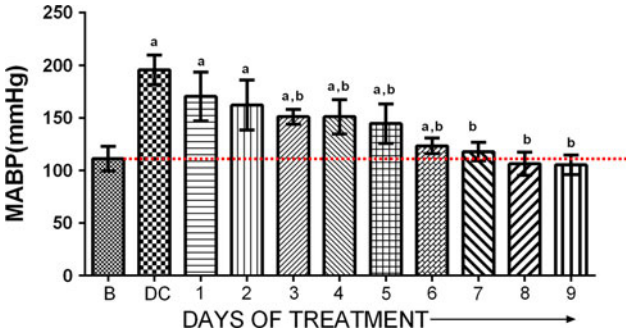

Fig. 3 Effect of high dose ALK on MABP in DOCA-treated rats: all values are expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus DOCA control. *B* basal value, *DC* DOCA control ($n = 5$)

Table 1 Effect of various pharmacological interventions on change in MABP in DOCA-treated rats

S. no.	Groups	Decrease in MABP (mmHg)	Basal MABP reached
1.	Low dose ALK	49	No
2.	Low dose AVE 0991	45	No
3.	High dose ALK	78	Yes
4.	High dose AVE 0991	81	Yes
5.	Combination of low dose ALK and low dose AVE 0991	82	Yes

All values are expressed as mean ± SD

ALK aliskiren

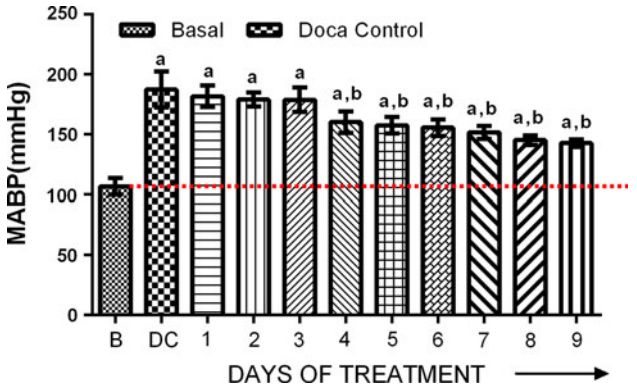

Fig. 4 Effect of low dose AVE 0991 on MABP in DOCA-treated rats: data is expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus DOCA control. *B* basal value, *DC* DOCA control ($n = 6$)

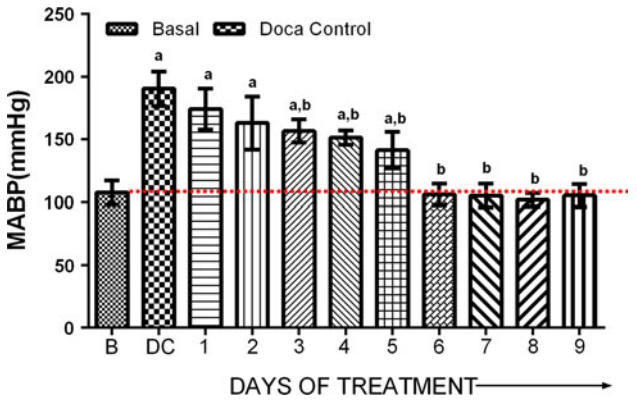

Fig. 5 Effect of high dose AVE 0991 on MABP in DOCA-treated rats: all values are expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus DOCA control. *B* basal value, *DC* DOCA control ($n = 6$)

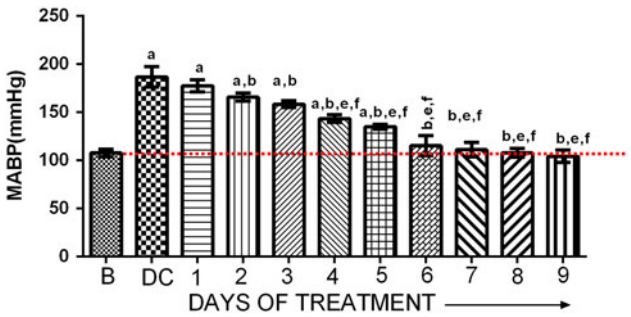

Fig. 6 Effect of combination of low dose of ALK and low dose of AVE 0991 on MABP in DOCA-treated rats: data is expressed as mean ± SD. *a* $P < 0.05$ versus basal; *b* $P < 0.05$ versus DOCA control; *e* $P < 0.05$ versus low dose ALK alone; *f* $P < 0.05$ versus low dose AVE0991 alone. *B* basal value, *DC* DOCA control ($n = 6$)

Effect of combination of low dose ALK and AVE 0991 on MABP in DOCA-treated rats

Treatment with combination of low dose ALK and AVE0991 significantly decrease MABP starting from day 2 and reached to the basal value on 8th day of treatment. The combination treatment significantly decreases MABP as compare with either drug alone in low doses (Fig. 6; Table 1).

Discussion

Principle findings of the study are that, treatment with combination of low doses of ALK and AVE 0991 produced synergistic antihypertensive effect in Wistar rats.

Chronic administration of DOCA–salt produces a progressive increase in systolic blood pressure and consequently, produced cardiac hypertrophy, and interstitial and perivascular fibrosis in uninephrectomized rats [26, 27]. In this study too, DOCA–salt-treatment resulted in significant increases in MABP in Wistar rats, which remained unchanged in the 2 week DOCA free period. It has been reported that, DOCA–salt HT is associated with up-regulation of the renal and cardiac RAAS [28]. Further, Tank et al. [29] found a significant increase in glomerular and proximal tubule renin mRNA in the remaining kidney after uninephrectomy. The intrarenally formed Ang II stimulates sodium and bicarbonate reabsorption possibly via activation of apical Na^+/H^+ exchange, basolateral Na^+–$HCO3^-$ cotransport, and basolateral Na^+/K^+–ATPase and via insertion of H^+–ATPase into the apical membrane and thereby regulates MABP in DOCA–salt-treated rats [30–35].

ALK is a new class of orally active, potent, low molecular weight, nonpeptide renin inhibitor [18, 19]. Renin inhibitors block the catalyzed hydrolytic cleavage of angiotensinogen by competitively binding to the active site and subsites of renin thus the generation of Ang I is inhibited and consequently less Ang II is available to maintain its biological effects. Further, ALK-reduced urinary aldosterone excretion and plasma aldosterone concentration by blocking plasma renin activity, reduces BP. Natriuresis was enhanced by ALK, whereas potassium excretion was not influenced. Reduction in aldosterone secretion can be regarded as a favorable neurohumoral effect of ALK, in light of the postulated detrimental pathophysiological role of aldosterone and the beneficial clinical effects of aldosterone antagonists in heart failure [36, 37]. Therefore, renin inhibitors have been suggested to provide more complete blockade of RAAS than ACEIs or ARBs [38, 39]. But it is likely that excessive inhibition of RAAS by renin inhibitor may also result in decreased formation of angiotensin (1–7) and may produced down-

regulation of Mas-receptor axis. Our study, for the first time, shows that combination of low doses of ALK with AVE 0991, a Mas-receptor agonist, produced synergistic antihypertensive effect in DOCA–salt-treated rats.

ACE2–Ang-(1–7)–Mas-receptor axis has been documented to play a role as an ACE–Ang II–AT1-receptor counter-regulatory axis [12]. It has been well-documented that beneficial effects of ACE-2/Ang(1–7)/Mas-receptor axis are abolished in hypertensive's, as ACE expression gets up-regulated and ACE-2 expression gets down-regulated leading to imbalance in two major axis of RAAS and consequent development of HT takes place [40]. Further, administration of Ang(1–7), a Mas-receptor agonist prevents development of severe HT and end-organ damage in spontaneously hypertensive rats (SHR) [25]. Furthermore, administration of Ang(1–7) has been shown to decrease the cardiac fibrotic changes associated with HT [38]. In this study, AVE0991, a Mas-receptor agonist produced antihypertensive effect for the first time in DOCA-treated Wistar rats in a dose-dependent manner.

After a single administration of ALK, peak plasma concentrations were reached in 1–2 h [38]. That may be sufficient for down regulation of angiotensin (1–7)/Mas-receptor counter regulating axis. Therefore, in this study, AVE 0991 was administrated 1 h after the ALK treatment in hypertensive rats.

Difference in the efficacy of angiotensin 1–7, a Mas-receptor agonist, in males and females rats have been reported earlier [41]. Therefore, we used animals of either sex in our study. But we did not find any sex-based differences in efficacy of AVE0991 in our study. However, our sample size ($n = 5$–6) is too small to contradict the results obtained by Sullivan et al. [41].

The result obtained in this study confirms the hypothesis that suppression of renin alone may result in down-regulation of ACE-2/Mas-receptor counter-regulatory axis of RAAS. Treatment with combination of low doses of ALK and AVE0991 for the first time, showed synergistic blood pressure lowering effect in DOCA–salt-treated rats. Therefore, combination of renin inhibitor with Mas-receptor agonist may prove beneficial for the treatment of hypertensive patients, than the effect of these drugs when used singly. Further research in this area is warranted.

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Conflict of interest None.

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