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Effect of demethylasterriquinone b1 in hypertension associated vascular endothelial dysfunction

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Abstract

Background: Activation of Akt stimulates phosphorylation of eNOS, production of nitric oxide and reduces oxidative stress. The study has been designed to investigate the effect of DAQ B1, an activator of Akt, in hypertension associated vascular endothelial dysfunction.
Methods: Rats were uninephroctomized and DOCA (40 mg kg^{-1}, *s.c.*) was administered to rats to produce hypertension (MABP > 140 mm Hg). Vascular endothelial dysfunction was assessed using isolated aortic ring preparation, electron microscopy of thoracic aorta and serum concentration of nitrite/nitrate. The expression of messenger RNA for *p22phox and eNOS* was assessed by reverse transcription-polymerase chain reaction. Serum TBARS and aortic superoxide anion were estimated to assess oxidative stress.
Results: DAQ B1 (5 mg kg^{-1}, *p.o.*) or atorvastatin (30 mg kg^{-1}, *p.o.*) markedly improved acetylcholine induced endothelium dependent relaxation, vascular endothelial lining, expression of mRNA for eNOS and p22phox, serum nitrite/nitrate concentration and serum TBARS in hypertensive rats. However, this ameliorative effect of DAQ B1 has been prevented by L-NAME (25 mg kg^{-1}, *i.p.*), an inhibitor of eNOS.
Conclusion: Therefore, it may be concluded that DAQ B1 induced activation of Akt may activate eNOS and consequently reduce oxidative stress to improve hypertension associated vascular endothelial dysfunction.

Keywords: Akt; Demethylasterriquinone b1; Hypertension; Vascular endothelial dysfunction

1. Introduction

Vascular endothelial dysfunction has been implicated in secondary complications due to essential hypertension [1], congestive heart failure [2,3], diabetes mellitus [4], hypercholesterolemia [5] and hyperhomocysteinemia [6]. DOCA induced hypertension [3] has been noted to produce experimental vascular endothelial dysfunction. The decrease in bioavailability, production and increase in metabolism of NO are considered to be responsible for endothelial dysfunction [2,7]. Akt activates phosphorylation of endothelial nitric oxide synthase [8] and stimulates NO production [2,9]. Activation of Akt reduces oxidative stress [10] Moreover, Akt increases protein synthesis by activating mammalian target of rapamycin and affects cellular metabolism by inhibiting glycogen synthase kinase-3 [11] Impaired membrane localization of Akt is noted to produce vascular endothelial dysfunction in hypertension [12]. DAQ B1 has been reported to be a selective activator of Akt [13–15]. It is reported from our laboratory that activation of Akt improves diabetes mellitus and hyperhomocysteinemia induced vascular endothelial dysfunction [16]. Therefore, the

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; CMC, carboxy methyl cellulose; DAQ B1, demethylasterriquinone B1; DMSO, dimethyl sulfoxide; DOCA, desoxycortisone acetate; eNOS, endothelial nitric oxide synthase; MABP, mean arterial blood pressure; NBT, nitroblutetrazolium; NO, nitric oxide; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of mean; SNP, sodium nitroprusside; TBARS, thiobarbituric acid reactive substances.

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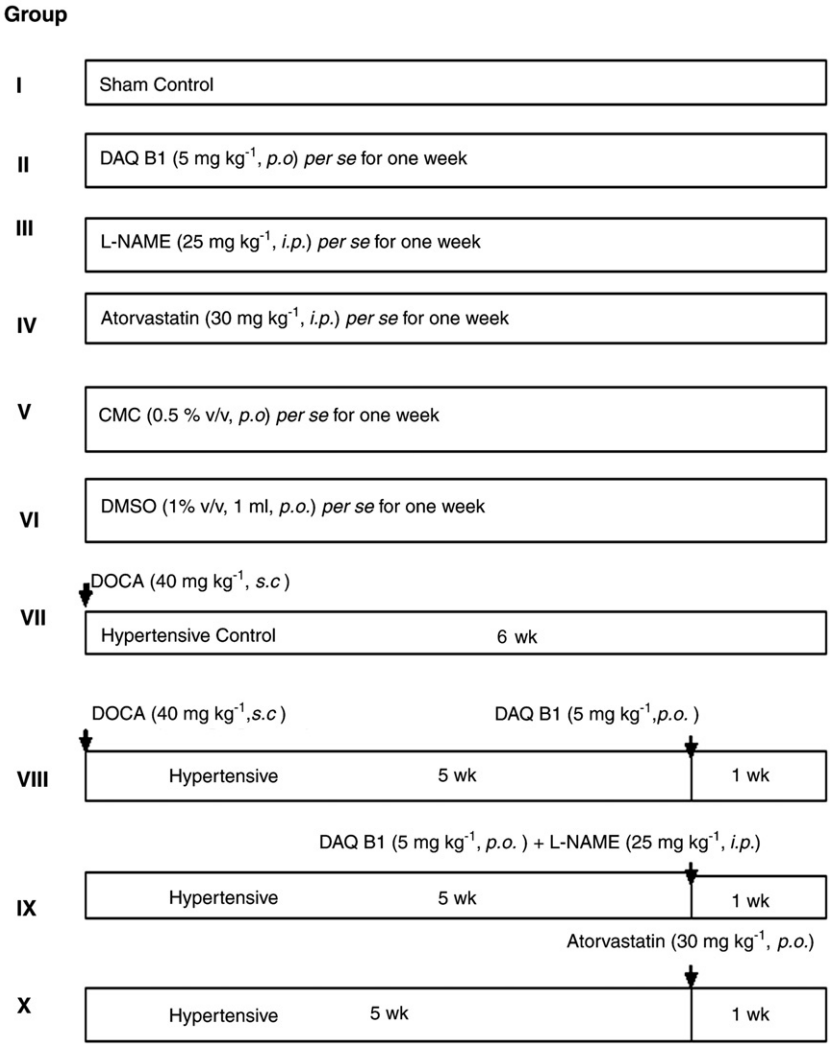

Fig. 1. Experimental protocol.

present study has been designed to investigate the effect of DAQ B1 in hypertension associated vascular endothelial dysfunction.

2. Materials and methods

Age matched young male *Sprague Dawley* rats weighing about 250–300 g were housed in animal house and were provided 12 h light and 12 h dark cycle. They were fed on standard chow diet (Ashirvad Agro India Ltd., Ambala, India) and were provided water *ad libitum*. Body weights and intake of fluid and food were measured daily during the study. The experimental protocol was approved by Institutional Animal Ethical Committee in accordance with guidelines of US National Institute of Health for Care and Use of Laboratory Animals. The rats were uninephroctomized and DOCA (40 mg kg^{-1}, *s.c.*) was administered twice a week up to six weeks to produce hypertension [3]. DOCA rats received 1.0% NaCl and 0.2% KCl in their drinking water. Sham and normal rats received tap water. Mean arterial blood pressure of rats was recorded by cannulating carotid artery with pressure transducer attached to BIOPAC Systems (BIOPAC, CA, USA).

2.1. Assessment of vascular endothelial dysfunction

2.1.1. Isolated rat aortic ring preparation

The rats were decapitated, thoracic aorta was removed, cut into rings of 4–5 mm width and mounted in organ bath containing Krebs–Henseleit solution (NaCl, 119 mM; KCl, 4.7 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.0 mM; glucose, 11.1 mM; KH_2PO_4, 1.2 mM and $CaCl_2$, 2.5 mM) bubbled with carbonated oxygen (95% O_2 and 5% CO_2) and maintained at 37 °C. The preparation was allowed to equilibrate for 90 min maintaining 1.5 g tension. The isometric contractions were recorded [17] with a force–displacement transducer (Ft-2147) connected to Physiograph (INCO, Ambala, India).

The preparation was primed with 80 mM KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of ACh (10^{-8} to 10^{-4} M) or SNP (10^{-8} to 10^{-4} M) were recorded in phenylephrine