Original Article

Evaluation of the coronary angiogenesis during DOCA-salt induced hypertension and its reversal to normal in rat

Mohammad Zarei[1], Majid Khazaei[2], Mohammad M. Zarei[3]

ABSTRACT

Objective: The aim of this study was to evaluate the effect of hypertension and its reversal to normal on coronary angiogenesis and serum vascular endothelial growth factor (VEGF) and its receptor (sFlt-1) concentrations in deoxycorticostrone acetate (DOCA)-salt induced hypertensive male rats.

Methodology: Forty male wistar rats were divided into four groups: 1) DOCA-salt; 2) Control-solvent; 3) DOCA-salt withdrawal; 4) Control-solvent withdrawal. Hypertension was induced by injection of DOCA together with oral salt-water for 12 weeks and then hypertension was reversed to normal by stopping DOCA injection for 12 weeks. Serum VEGF and sFlt-1 concentrations and heart coronary capillary density were measured before experiment, after induction of hypertension and after DOCA-salt withdrawal.

Results: In DOCA-salt group, serum VEGF level was significantly decreased compared with normotensive rats while serum sFlt-1 concentration was significantly increased. In DOCA-salt withdrawal group, serum VEGF level was returned to normotensive level but not to a significant extent and serum sFlt-1 level was also significantly decreased. In hypertensive rats, heart coronary capillary density was lower than normotensive rats and after returning of hypertension to normal it was changed toward normotensive level (no significance).

Conclusion: Hypertension changed serum VEGF and sFlt-1 concentrations and return of hypertension to normal caused a reversal of serum VEGF and sFlt-1 concentrations at least in this model. It shows that early diagnosis and treatment of hypertension is important in clinical condition.

KEY WORDS: Hypertension, Deoxycorticosteron Acetate, Angiogenesis, Coronary, Vascular endothelial growth factor.

Abbreviation: DOCA, Deoxycorticosteron Acetate; VEGF, Vascular Endothelial Growth Factor; sFlt-1, Vascular Endothelial Growth Factor Receptor-1.

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INTRODUCTION

Endothelial dysfunction which is an early symptom of the development of cardiovascular disorders is related to hypertension and atherosclerosis and may be associated in bringing about the vascular complication of hypertension.[1,2] Angiogenesis is a procedure that new vessels grow from previous vessels in reaction to antigenic molecules effect and hypoxia that result from tissue damage. Moreover, angiogenesis is an important process in development of circulatory system. Actually, VEGF has a key role in physiological and pathological vascular development among the angiogeneic factors.[3]

VEGF is a 45-kDa glycoprotein which stimulates proliferation and migration of endothelial cells and inhibits apoptosis resulting in formation of collateral vessels.[4] VEGF has two tyrosine kinase receptors: VEGR-R1 (sFlt-1) and VEGF-R2. sFlt-1 is soluble form which is found in circulation and can inhibit VEGF actions by direct sequestration.[5-7] It is well known that hypertension along with several vascular disorders like endothelial dysfunction[8], microvascular rarefaction and remodeling is a serious risk factor..[9,10] It has been proposed that hypertension defects blood vessel growth.[11,12] Some of the studies show that impaired angiogenesis is in hypertensive subjects[13], but other studies have reported increased angiogenic factors in serums of hypertensive subjects.[14-16] Never the less, impaired angiogenesis leads to underdevelopment of vascular system and predispose hypertension in later life.[11]

We have previously reported the study of coronary angiogenesis in two kidney Goldblatt hypertension and DOCA-salt hypertensive ovariectomized models.[17,18] However, the effect of hypertension on coronary angiogenesis in deferent hypertension models is very complex and is not completely understood. The main aim of this study was to evaluate the effect of hypertension and its reversal to normal on coronary angiogenesis and serum VEGF and sFlt-1 concentrations in DOCA-salt induced hypertensive male rats.

METHODOLOGY

Animals and experimental groups: A total of 40 male wistar rats (200±20 gr) were purchased from Pasteur Institute of Tehran, Iran. They were housed in an animal room at 22–24 ^{o}C and given free access to tap water and rat chow. The animals were kept in 12:12 hr. light: dark round (light period 08.00–20.00 hr.). All the experimental procedures including rat care and handling were employed, which were in agreement with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care Committee of Isfahan University of Medical Sciences.

After one week habituation of animals to condition of animal room, first they were divided into two groups: DOCA-salt and Control-solvent. After induction of hypertension in DOCA-salt group for 12 weeks, either of two groups of DOCA-salt and Control-solvent was divided into two groups as follow:
Group 1 (n=10): DOCA-salt for 12 weeks
Group 2 (n=10): Control-solvent for 12 weeks
Group 3 (n=10): DOCA-salt withdrawal for 12 weeks (from among DOCA-salt induced hypertensive rats after 12 weeks).
Group 4 (n=10): Control-solvent withdrawal for 12 weeks (from among Control-solvent rats after 12 weeks).

In groups 1&2 (n=10 each), the animals were killed and serum VEGF and sFlt-1 levels and coronary capillary density were measured. In groups 3&4 (n=10 each), DOCA injection was stopped and serum VEGF and sFlt-1 levels and coronary capillary density were also measured after 12 weeks of reversal of hypertension.

Induction of DOCA-Salt model of hypertension and its reverse to normal: The animals were anesthetized by ketamin (75 mg/kg; i.p) and xylazine (7.5 mg/kg; i.p). All rats underwent uninephrectomy via left flank incision. Then, the wounds were closed with silk suture. After recovery, DOCA-salt group received subcutaneously injection of DOCA (30 mg/kg), twice a week and sodium chloride solution (1%) instead of tap water, Control-solvent group received subcutaneously vehicle of DOCA with the same volume plus tap water throughout the experiment. For reversal of hypertension to normal in hypertensive group, injection of DOCA was completely stopped and the animals received tap water instead of NaCl 1% solution.[19,20]

Blood pressure measurement: The animals were kept in a temperature-controlled restrainer and indirect systolic blood pressure was measured every week using tail pressure cuff which was connected to a power lab signal transduction system and associated chart software (AD Instrument, USA). Carotid artery was cannulated by using of PE-50 catheter and direct blood pressure was also measured 12 weeks after injection of DOCA in groups 1 and 2. After stoppage of DOCA injection in groups 3&4, indirect blood pressure was measured every week and after 12 weeks, direct blood pressure was measured as previously described. The average systolic blood pressure of direct and indirect measurement was expressed.

Serum VEGF and sFlt-1 measurements: By using ELISA method, a Sandwich Enzyme Immunoassay kits and reagents (R&D systems, USA), serum VEGF and sFlt-1 assays were measured according to its manufactures' instruction. The method for measuring of VEGF has a minimum sensitivity of 3.9 pg/ml with an intra- and inter assay coefficient of variation (CV) of <10% and <5%, respectively but the sFlt-1 assay has lower limit of sensitivity of 3.8 pg/ml and intra- and inter assay coefficients of variation less than 10% and 5%, respectively.

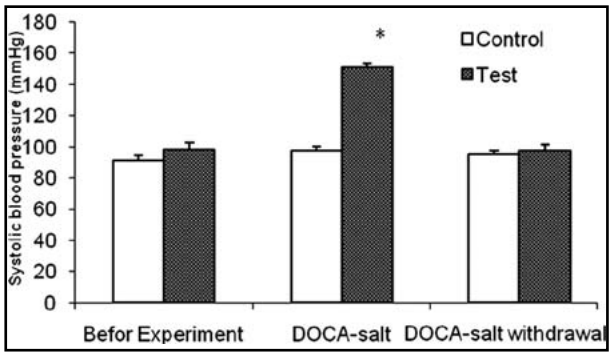

Fig.1: Systolic blood pressure before experiment, after induction of hypertension and after DOCA-salt withdrawal (*p < 0.05 when compared to other groups).

Evaluation of capillary density: We used apex of heart as tissue samples after 12 weeks induction of hypertension and then its reversal to normal. Frozen tissue sections with 5 µm thickness were prepared from each sample. Endothelial cells were identified by immunohistochemical staining using rat anti-mouse CD31 monoclonal antibody (Abcam Co, USA). Capillary density was evaluated by counting twenty random microscopic fields (magnification×400) from three different sections in each tissue block by three blind examiners. Capillary density was expressed as the number of $CD31^+$ cells per square millimeter (mm^2).

Statistical Analysis: Data are reported as mean ± S.E. One-way ANOVA was used for comparison of data between groups. The difference between two groups was analyzed by student's t-test. Data before and after experiment were compared by pair t-test. p-values less than 0.05 were considered statistically significant.

RESULTS

Blood pressure: Results showed that in animals that received DOCA-Salt treatment, blood pressure increased gradually during three weeks and maintained at that level in the next weeks (data not shown). Fig.1 illustrates systolic blood pressure before experiment, 12 weeks after DOCA-salt treatment and 12 weeks after DOCA-salt withdrawal. As shown in Fig.1 animals that received DOCA-salt treatment, systolic blood pressure was significantly increased compared with control group (151.45 ± 2.18 vs. 97.7 ± 2.32 mmHg; p<0.05). Withdrawal of DOCA-salt almost completely reversed systolic blood pressure in this group (98.01 ± 3.67 vs. 151.45 ± 2.18 mmHg, respectively; p<0.05).

Serum VEGF and sFlt-1 concentrations: Serum VEGF levels was significantly reduced in hypertensive rats with mean concentration of 83.43± 5.56 pg/ml compare with normotensive group (98.48±2.18 pg/ml) (p<0.05). With return of hypertension to normal in DOCA-salt withdrawal group, serum VEGF concentration was slightly increased close to normotensive level but not to a significant extent (Fig.2a). Serum sFlt-1 level was increased after induction of hypertension (3431.01 ± 162.71 vs. 599.96 ± 91.78 pg/ml, respectively; p<0.05). However, reversal of blood pressure to normal decreased serum sFlt-1 concentration to DOCA-salt level (1658.5 ± 283.31 vs. 3431.01 ± 162.71 pg/ml; p<0.05) (Fig.2b).

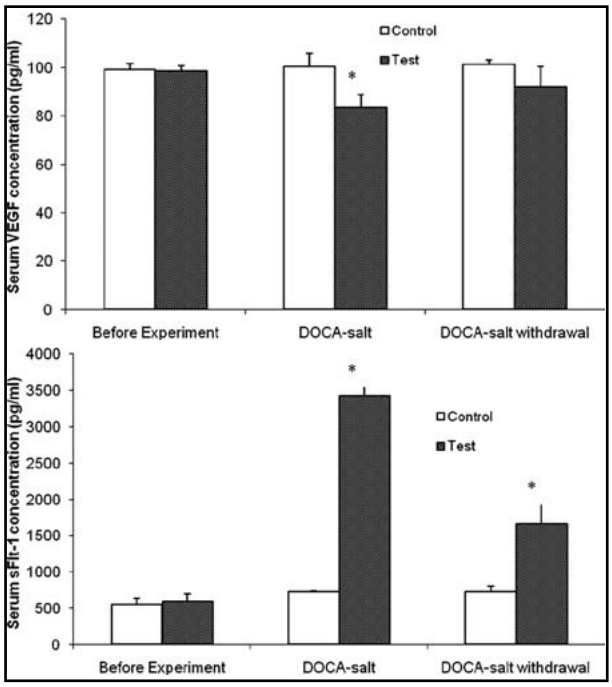

Fig.2: Serum VEGF (a) and sFlt-1 (b) concentrations before experiment, after induction of hypertension & after DOCA-salt withdrawal (*p < 0.05 when compared to other groups).

Capillary density: As demonstrated in Fig.3, capillary density (expressed as number of capillary ($CD31^+$) per mm^2) was lower in DOCA-salt

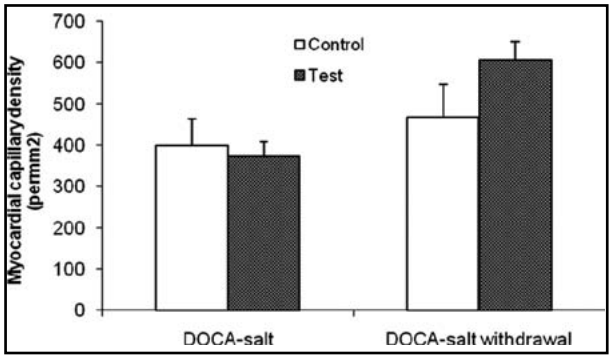

Fig.3: Capillary density (expressed as number of capillary/ mm2) of heart (apex) after induction of hypertension and DOCA-salt withdrawal in male rat.

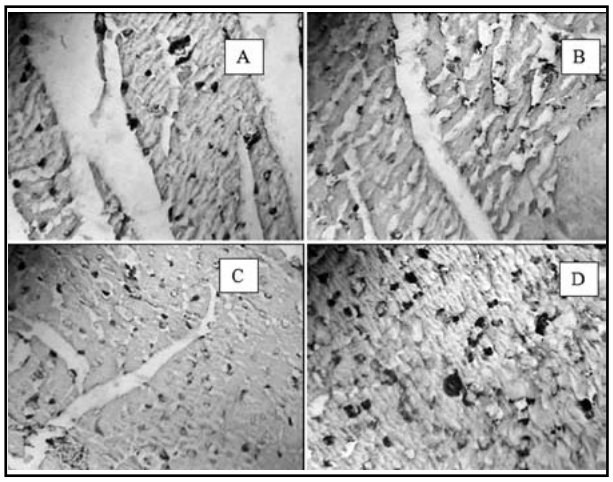

Fig.4: Representative photograph of the cross section of apex of the hearts in experimental groups. Original magnification: ×400. (A: Control- solvent; B: DOCA-salt; C: Control-solvent withdrawal; D: DOCA-salt withdrawal).

hypertensive animals normotensive group, but it wasn't statistically significant. In DOCA-salt withdrawal group, capillary density was raised compare with normotensive animals. Samples of histological sections are shown in Fig.4.

DISCUSSION

The data presented in this study show that changes in serum VEGF and sFlt-1 concentrations in DOCA-salt induced hypertension are reversible by removing the cause of hypertension. But these findings did not show any significant differences between groups in capillary density. Hypertension is associated with several vascular abnormalities including endothelial dysfunction and vascular rarefaction which could contribute to increased vascular resistance and hypertension.[21-24] Little is known about rat coronary angiogenesis so based on results of this study, coronary angiogenesis dropped in hypertensive rats but not to a significant extent.

Literature review showed that changes of micro vascular density during development of hypertension has been documented in several experimental studies.[17,18,25-26] A study in SHR rats showed higher vessel density in mesentery compare with similar area in normal rats.[26] Another study in hypertensive rats showed lower capillary and arteriolar density in the heart of young animals.[25] An interesting study demonstrated that in human, no changes were found in capillary number of quadriceps muscle of hypertensive subjects.[27] Of the many evidences, it seems that effect of hypertension on microcirculation depends on tissue, time of developing hypertension and model of hypertension.[11,26]

It is well-known that VEGF is very important in endothelial and vascular function. Earlier long-term studies has documented that VEGF stimulates endothelial migration, proliferation, and survival and enhances nitric oxide production.[29-31] It also inhibits endothelial apoptosis. VEGF-R1 is a tyrosine kinase receptor which expressed primarily on endothelial cells and involves in regulation of angiogenesis, endothelial cell migration and proliferation.[29] It is interesting to note that for first time, in 1995, Fong et al. revealed that VEGF-R1 plays a negative role in angiogenesis[32] VEGF-R1 has high affinity for VEGF but has weak tyrosine kinase activity. It is indicated that soluble VEGF-R1 (sFlt-1) has anti angiogenic properties by dampening angiogenic VEGF-VEGFR2 signalling.[6,7,33,34]

In this study, we found that serum VEGF level was decreased during hypertension and reversal of hypertension returned it to near normotensive level but sFlt-1 level was dramatically increased during hypertension and reversal of hypertension decreased it. There are contradictory reports regarding the changes of plasma angiogenic factors during hypertension. Belgore et al showed that plasma VEGF and sFlt-1 concentrations were significantly higher in uncomplicated essential hypertensive patients compared with normotensive controls and treatment of hypertension significantly reduced plasma VEGF and sFlt-1 levels.[14-16]

Other clinical studies demonstrated a positive association of VEGF and HGF with hypertension.[5,21-23] They suggested VEGF may be a marker for hypertension with a response to high blood pressure. In contrast, Felmeden et al reported higher VEGF and lower sFlt-1 in plasma of hypertensive patients.[23]

A recent study has indicated that plasma VEGF and sFlt-1 levels have no correlation with blood pressure.[35] These data variability can be attributable to methodological differences, sampling preparation and method of analysis of these factors. It should be considered that method used to determine free VEGF, total VEGF or total VEGF-VEGFR1 complex (bound or unbound proteins) makes a difference in quantification of circulating VEGF or sFlt-1 levels in studies.[6,7]

In conclusion, our result showed that changes in serum VEGF and sFlt-1 concentrations in DOCA-salt hypertension reversed by removing the cause of hypertension. However, there isn't any significant difference between heart capillary density before and after DOCA-salt hypertension.

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