

Anti-Hypertensive Effect of Water Extract of Danshen on Renovascular Hypertension Through Inhibition of the Renin Angiotensin System

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Abstract: A study was designed to elucidate the mechanism of anti-hypertensive effects of Danshen in the two-kidney, one clip (2K1C) Goldblatt renovascular hypertensive model, which is the renin-angiotensin system (RAS)-dependent hypertensive model. We investigated the effects of water extracts of Danshen on the angiotensin converting enzyme (ACE) activities, systolic blood pressure (SBP), and hormone levels in the plasma of 2K1C rats. ACE activity was inhibited by the addition of Danshen extract in a dose-dependent manner. SBP was decreased significantly after administration of Danshen extract in 2K1C, whereas plasma renin activity (PRA) was not changed. The plasma concentration of aldosterone (PAC) was decreased significantly in 2K1C group administered with Danshen extract, whereas the plasma concentration of ANP was increased by administration of Danshen extract for three weeks. These results suggest that Danshen has an anti-hypertensive effect through the inhibition of ACE, an essential regulatory enzyme of RAS.

Keywords: Danshen Extract; Angiotensin Converting Enzyme; Hypertension.

Introduction

The renin-angiotensin system (RAS) plays a dominant role in the regulation of the water electrolyte balance and blood pressure. Activation of this system has been considered to be a main cause of renovascular hypertension (Schricker *et al.*, 1994). The two-kidney, one clip (2K1C) Goldblatt hypertensive rat is a useful animal model for the study of renovascular hypertension, which is a RAS-dependent hypertensive model. Angiotensin converting enzyme (ACE) is the most important regulatory site of RAS. The major physiological function

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of ACE is linked to the regulation of blood pressure and electrolyte homeostasis by converting angiotensin I (Ang I) into potent vasoconstrictor angiotensin II (Ang II) and by inactivating bradykinin (Johnston *et al.*, 1979). The importance of ACE inhibitors in the chronic treatment of various cardiovascular diseases such as hypertension, congestive heart failure, myocardial infarction, diabetic nephropathy, or renal dysfunction is now well established. In fact, inhibitors of ACE such as Captopril, Enalapril, Lisinopril and Temocapril are widely used in the clinic for the treatment of hypertension. Moreover, variations of serum ACE activity have been reported in pathologies involving either a stimulation of monocyte cell line or an endothelial abnormality (Papapetropoulos *et al.*, 1996).

Danshen, roots of *Salviae miltiorrhiza*, have been used in Chinese folk medicine for the treatment of coronary heart diseases, myocardial infarction and hypertension. It has been reported that many components such as pigments and some phenolic compounds, including lithospermic acid B are involved in these crude drugs (Fung *et al.*, 1993). Nagai *et al.* (1996) and Kamata *et al.* (1993 and 1994) have demonstrated that Danshen water extract was able to dilate the rat aorta in an endothelium-dependent manner. On the other hand, Li *et al.* (1990) suggested that the anti-hypertensive effect of Danshen is angiotensin- and/or bradykinin-related in the periphery and on the heart via muscarinic receptors. However, there is no further evidence to support this suggestion. Nonetheless, although it is well known that Danshen has a depressive effect in hypertension, the mechanism of this action has not yet been made clear. For these reasons, in the present study, we tested the hypothesis that Danshen has a depressive effect through the inhibition of ACE. To clarify this hypothesis, we first assayed ACE inhibitory activities of Danshen extract. Second, we evaluated the effects of Danshen extract not only on blood pressure, but also PRA, PAC and ANP of 2K1C hypertensive rats to elucidate the mechanism of the depressor effect.

Materials and Methods

Plant Material

Danshen was purchased from the local herb drug market and identified taxonomically. A Danshen sample (300 g) was extracted with 1.5 liters of distilled water for 120 minutes at 100°C. The water extracts were then centrifuged (4°C, 3000 rpm, minutes) and filtered through Whatman No. 3 filter paper. After filtration, it was dried using a rotary vacuum evaporator (R110, Buchi, Schweiz, Switzerland) at 60°C for 3 hours. Dried Danshen extracts were resuspended in 300 ml distilled water and stored at -20°C until use.

Experimental Animals

Male Sprague-Dawley rats were purchased from Samyuk (Samyuky, Osan, Korea) and used in this study. All animals were fed *ad libitum* with water and commercial laboratory chow, Samyuk#31 (Samyuky, Osan, Korea). The experimental animals were kept at a vivarium with light-dark cycles of 12-hour/12-hour. Two-kidney, one clip Goldblatt hypertensive rats (2K1C) were prepared from male Sprague-Dawley rats, following the

reported procedures (Lee *et al.*, 1993). Animals were anesthetized with Nembutal (i.p., 30 mg/kg), followed by making a 3 cm incision on the left flank parallel to the left paramedian line. The abdominal viscera were then opened and separated by light traction. After separation procedures, a silver clip (0.22 mm) was applied to the left renal artery. Danshen extracts were administered 150 μ l/kg of body weight. Systolic blood pressure (SBP) was measured in a conscious state by the tail-cuff method.

On the day of the experiment, SBP was measured, and the trunk blood was collected by decapitation without anesthesia in a prechilled tube, containing 5 mg/ml of ethylenediaminetetraacetic acid (EDTA), soybean trypsin inhibitor (SBTI, 50 BAEE/ml) and aprotinin (200 KIU/ml), for determination of plasma ANP, rennin activity and aldosterone concentration.

Determination of ACE Activity

ACE activity was determined in rat plasma by the method described by Santos *et al.* (1985). Briefly, plasma (10 ml) were incubated with 490 or 480 μ l of assay buffer containing 5 mM Hip-His-Leu in 40 mM sodium borate buffer and 0.9 M NaCl, pH 8.3, for 15 minutes at 37°C. The reaction was stopped by the addition of 1.2 ml of 3.4 N NaOH. The product, His-Leu, was measured fluorimetrically at 365 nm excitation and 495 nm emission with fluorescence spectrophotometer (Hitachi, model F-2000, Tokyo, Japan) as follows. One hundred μ l of o-phthalaldehyde (20 mg/ml) in methanol was added and after 10 minutes, the solution was acidified with 200 μ l 3 N HCl and centrifuged at 3000 rpm for 10 minutes at room temperature. To correct for the intrinsic fluorescence of the plasma, time zero blank was prepared by adding plasma after NaOH.

Assay of Plasma Renin Activity, ANP and Aldosterone Concentration

Plasma renin activity was determined by radioimmunoassay (RIA) of angiotensin I (AI) (Lee *et al.*, 1991) and expressed as nanogram Angiotensin I per milliliter per hour (ng Ang I/ml/hr). Plasma atrial natriuretic peptide was measured by radioimmunoassay as described previously (Cho *et al.*, 1988). Plasma aldosterone concentration was measured by an RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA).

Drugs

Drugs were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Statistical Analysis

Results were expressed as mean \pm S.E.M. The statistical significance of differences between the groups was determined using Student's t-tests.

Results

Figure 1 shows *in vitro* ACE activity changes in the plasma by addition of Danshen extract (400 $\mu\text{g/ml}$). The ACE activities were decreased significantly by addition of Danshen extract (Fig. 1A). The responses were dose-dependent (Fig. 1B). The ID_{50} for ACE-inhibition of Danshen extract was approximately 170 $\mu\text{g/ml}$. Figure 2 shows the effects on the SBP of 2K1C hypertensive rats during the three-week period of administration of Danshen extract. At the starting point of experiment (three weeks after clipping), the SBP in 2K1C rats was increased in 157 ± 5 mmHg. The increase of SBP in 2K1C rats was sustained for the whole experiment period, whereas the administration of Danshen extract during whole experiment prevented the increase in SBP. Following the three-week administration of Danshen extract in the 2K1C rats, plasma rennin activities, measured by RIA, did not differ between the groups: 21.2 ± 4.2 ng Ang I/ml/hr in 2K1C group, 22.4 ± 6.1 ng Ang I/ml/hr in 2K1C administrated with Danshen extract (Fig. 3A). The plasma concentration of aldosterone was decreased significantly in Danshen extract administrated 2K1C group compared with their control 2K1C group (2K1C: 82 ± 11 , 2K1C group administrated with Danshen extract: 46 ± 8 pg/ml, Fig. 3B). In contrast, the plasma concentration of ANP was increased by administration of Danshen extract for three weeks compared with control group (2K1C: 102 ± 12 , 2K1C group administrated with Danshen extract: 162 ± 16 pg/ml, Fig. 3C).

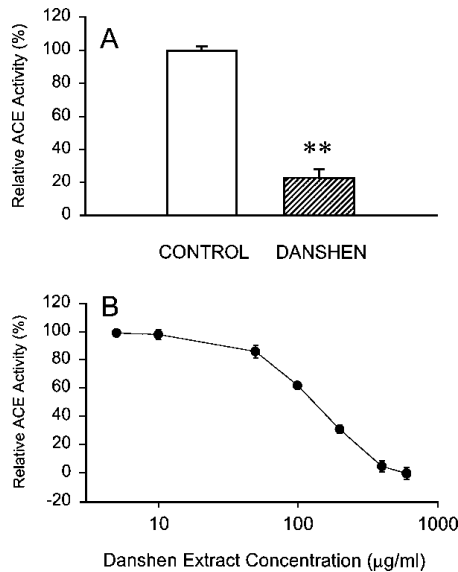


Figure 1. Effects of Danshen extract on plasma ACE activities (A) and dose-dependent inhibition of Danshen extract on ACE activities (B). 400 $\mu\text{g/ml}$ of Danshen extract was added in each ACE assay mixture (A). Each data shows mean \pm SEM of four experiments (** $p < 0.01$, compared with control).

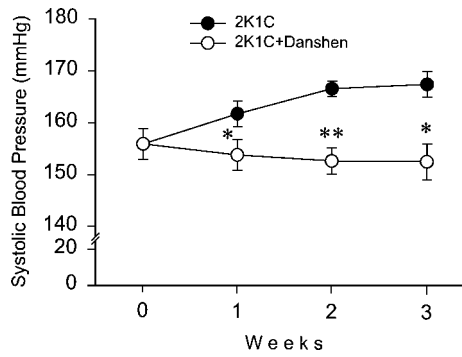


Figure 2. Systolic blood pressure changes in 2K1C and Danshen water extract-administrated 2K1C rats. Danshen extract (150 $\mu\text{g/ml}$) was administered during the 3 weeks. There are seven experiments in each group (* $p < 0.05$, ** $p < 0.01$, compared with 2K1C group).

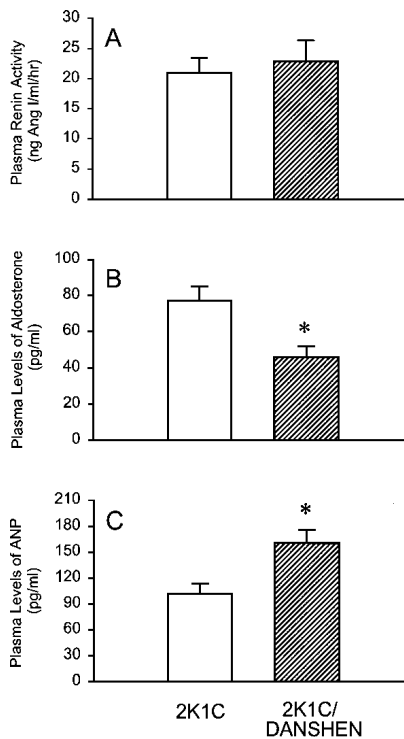


Figure 3. Effects of Danshen extract on plasma renin activities (A), aldosterone (B), and ANP (C) levels. Danshen extract (150 $\mu\text{g/ml}$) was administered during the 3 weeks. Each column shows mean \pm SEM of seven experiments (** $p < 0.05$).

Discussion

The present study showed an anti-hypertensive effect of Danshen extract in 2K1C renovascular hypertension. In the early 1930s, Goldblatt described a model of renovascular hypertension in dogs, and later, Miksche *et al.* (1970) established the 2K1C models in rats. This model was used extensively for a better understanding of the relationship among the RAS, hypertension and cardiovascular disorder. Irrespective of species, the 2K1C model is characterized by an increase in blood pressure immediately after clipping, which parallels the release of active renin concentration. The contribution of the RAS to the development and maintenance of 2K1C hypertension has been well established (Schricker *et al.*, 1994). The plasma and renal renin levels were found to increase in both the early and chronic phase of 2K1C hypertensive rats (Michel *et al.*, 1986; Schricker *et al.*, 1994). The ACE is the most important regulatory site of RAS. The ACE plays an important role in the regulation of blood pressure and diuresis. It catalyzes the hydrolysis of Ang I to Ang II, which is a powerful vasoconstrictor. Ang II also increases the synthesis and release of aldosterone, which cause sodium and water retention (Rang and Dale, 1987). Recently, it has been demonstrated that the inhibitor of ACE was the most effective anti-hypertensive drugs in RAS-dependent hypertension. The important findings in this study were that Danshen extract has an inhibitory effect on ACE. Furthermore, Danshen extract blocked the increase of blood pressure in 2K1C hypertensive rats. These results suggest that Danshen has an antihypertensive effect via the inhibition of ACE, an essential regulatory enzyme of RAS. The results of the present study in the plasma aldosterone concentration supported this suggestion. Where RAS was up-regulated in renovascular hypertension, the synthesis and release of aldosterone is suppressed by the inhibition of ACE. On the other hand, plasma concentration of ANP was increased in 2K1C rats administrated with Danshen extract compared with 2K1C control group. It is likely that the increased plasma ANP concentration may also contribute to the depressor effect on blood pressure 2K1C rats administrated with Danshen extract.

Taken together, our results indicate that Danshen extract has a depressor effect in 2K1C renovascular hypertension, which is the RAS-dependent hypertensive model, via inhibition of ACE.

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