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Review article

Mechanisms of acupuncture and herbal medicine in hypertension

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Background: Acupuncture and herbal medicine have been of great benefit to the Asian people for centuries. The discipline has however not been subjected systematically and thoroughly to the rigors of scientific testing.
Objective: This article reviews experimental evidence in regards to the vascular mechanisms of acupuncture and herbal medicine in hypertension. The focus is on our hypothesis that acupuncture and herbal medicine reduce hypertension through activation of microvascular endothelial nitric oxide synthesis (eNOS). We also examine whether or not our results in experimental renovascular hypertension conform to the meridian theory.
Results and conclusion: Acupuncture and herbal medicine 1) reduce experimental renovascular hypertension; 2) increase production of nitric oxide (NO), and contribute to vasodilation in the microvasculature and reduction of peripheral vascular resistance. It was concluded that acupuncture and herbal medicine target eNOS and activate its signaling mechanisms, and that the benefits of acupuncture proceeds along the meridian of the stimulated acupoint.

Keywords: Acupuncture, complementary and alternative medicine, endothelial nitric oxide synthase, hypertension, meridian theory, nitric oxide, Radix Salvia miltiorrhizae, Ramulus cum Uncis Uncariae.

Acupuncture and herbal medicine have been of great benefit to the Asian people for centuries. Complementary and alternative medicine (CAM) is a term that includes acupuncture and herbal medicine. It has greatly expanded its influence and is embraced today by nearly one in three adults in the USA and European countries, and at a much higher rate in Asian countries [1-3]. The general public and the traditional medical establishment are interested in CAM now more than ever. This discipline has however not been subjected systematically and thoroughly to the rigors of scientific testing.

Hypertension affects millions of people in the world. It causes loss of lives as well as having an economic impact. Further, some therapeutic regimens currently available for treating hypertension have their own significant side effects. Thus, it is important to investigate the benefits of acupuncture and herbal medicine in the treatment of hypertension in order to obtain greater success in either curing or controlling this silent killer, and to minimize its side effects.

This article reviews experimental evidence of the vascular mechanisms of acupuncture and herbal medicine in hypertension, focusing on our hypothesis that acupuncture and herbal medicine reduce hypertension through activation of microvascular endothelial nitric oxide synthesis.

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Acupuncture and hypertension

Acupuncture has been used in Asia for centuries to treat hypertension. Acupuncture points of Shixuan, stomach (ST)-36, liver (LIV)-3, gall bladder (GB)-20, GB-34, large intestine (LI)-4, LI-11, urinary bladder (UB)-15, UB-20, UB-23, and spleen (SP)-6 have been used traditionally for the treatment of hypertension [4].

Acupuncture points were initially thought to apply exclusively to humans because of anatomic discrepancies between humans and animals (size, biped standing). Remarkably, a 79 % correlation exists between canine lower electrical skin resistance and impedance points (electrically active skin points) and human acupuncture points [5]. Small animals have at least 142 acupuncture points and share the meridian

system with humans [6]. The canine acupuncture atlas shows 136 acupuncture points that are effective for small animal acupuncture [7].

Several acupuncture points, such as stomach (ST)-36, liver (LIV)-3, large intestine (LI)-4, and Shixuan point, have been established in animal models, including hamsters and mice. The ST-36 point has been studied in a) neuronal NOS related to cardiovascular regulation [8, 9], b) efficacy of analgesia [10, 11], c) modulation of immune activity [12], d) neuronal mechanisms [13-15], e) treatments of colitis, rheumatoid arthritis and liver injury [16-18], and f) effects on alcohol-drinking behavior, depression-like behavior, gastric motility, diabetes, and cell proliferation [19-23]. Recently, we demonstrated in hamsters that acupuncture on ST-36 reduces blood pressure in experimental renovascular hypertension [24]. LIV-3 point has been studied in a) treatment of liver injury (18), b) neuroprotective effects against neuronal death in the rat Parkinson's disease model [25], c) uterine contractions [26], and d) hypertension [27]. LI-4 point has been studied in a) efficacy of analgesia [28], b) blood pressure and nerve activity [29 30], c) treatment of colitis (16), d) autonomic nerve activity [31] and e) uterine contraction [32]. Shixuan point has been studied for anti-hypertensive effects in two-kidney one-clip Goldblatt hypertensive rats [33].

Acupuncture influences hormones related to the regulation of blood pressure and body fluid metabolism. Acupuncture at urinary bladder (UB)-15 point increases plasma levels of atrial natriuretic peptide, and acupuncture treatment at UB-20 point decreases the plasma levels of renin activity and atrial natriuretic peptide [34]. Acupuncture on ST-36 and gall bladder (GB)-34 decreases blood pressure in hypertensive patients [35]. Acupuncture on Shixuan point decreases both systolic blood pressure and plasma renin activity [33]. These results suggest that the beneficial results are due to a decrease in renin secretion. The preceding studies focused on reduction of factors that elevate blood pressure, such as the renin-angiotensin system. We [24, 36] and others [8] have recently directed attention to mechanisms that enhance vasodilation and bradycardia as an alternative approach to understanding the impact of alternative medicine in hypertension. These studies support the application of human acupuncture points to animals, and open opportunities for the systematic studies of the mechanisms of acupuncture.

Acupuncture reduces blood pressure in hamsters

We chose the two-kidney 1-clip (2K1C) model of renovascular hypertension [33, 37] to investigate the efficacy of acupuncture using male golden Syrian hamsters as an animal model [38, 39]. We tested whether or not acupuncture on Shixuan point (located on the tips of each forepaw, just below nails, 5 points on each side) and Stomach 36 point (ST-36, located on the outside of the hind leg, just below the knee, and outside of the tibial crest, in the middle of the cranial tibial muscle belly [8, 24] would have beneficial effects and lower blood pressure. Our study groups included 2K1C hypertension hamsters without acupuncture, 2K1C hypertension hamsters with acupuncture, sham-operated hamsters without acupuncture, and sham-operated hamsters with acupuncture. We performed manual acupuncture on Shixuan point and electroacupuncture on ST-36 (with model KWD-808II, Shenzhen Kuanyu Electronic Co, Shenzhen, China). During the manual acupuncture treatment, hamsters were restrained from the back for application of the acupuncture treatment. Shixuan point was pricked quickly with a single stimulation. The acupuncture needle (diameter 0.20 mm) did not remain on the body. During the administration of electroacupuncture, the hamster was restrained in a Ballman cage (Natsume Seisakusho Co, Tokyo, Japan).

We started the acupuncture treatment two weeks after surgery and continued it for two additional weeks for manual acupuncture on Shixuan point and 5 days for electroacupuncture on ST-36. The mean arterial pressure (MAP) in the control sham-operated group was 115.2 7.6 mmHg. Renovascular hypertension was demonstrated in the 2K1C group by elevation of MAP to 162.4 7.1 mmHg in 2K1C hamsters relative to the non-treated sham-operated hamsters. The manual acupuncture on Shixuan point significantly reduced MAP to 144.0 4.3 mmHg in 2K1C hamsters, while electroacupuncture ST-36 brought MAP to 128.0 4.3 mmHg in 2K1C hamsters (**Fig. 1**). These results verify that manual acupuncture and electroacupuncture produce similar beneficial effects on blood pressure reduction in hypertensive animals.

To determine whether the acupuncture effect was placebo-like, we performed acupuncture on Shixuan and ST-36 on sham-operated normotensive hamsters. There was no significant mean arterial blood pressure difference between sham-operated hamsters with acupuncture treatments and those without acupuncture treatments. These data suggest that the

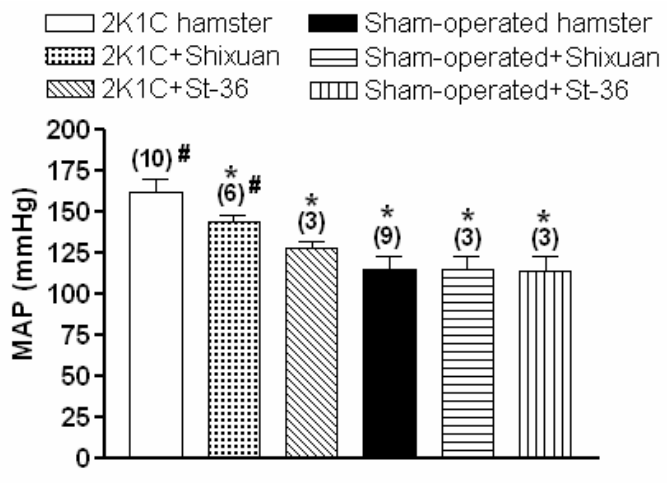

Fig. 1 Effects of manual acupuncture on Shixuan point and electroacupuncture on ST-36 point on mean arterial blood pressure (MAP) after induction of hypertension. The bars represent the mean SEM. The numbers in parenthesis show the number of hamsters in each group. *p<0.05 compared with non-treated renovascular hypertensive (2K1C) hamsters, #p<0.05 compared with sham-operated hamsters. (Modified from [24]; reproduced with permission).

efficacy of acupuncture in the management of blood pressure is manifested only when arterial pressure is elevated. This observation is in agreement with a report that acupuncture treatment reduces systolic blood pressure in 2K1C hypertensive rats, but not in sham-operated rats [33]. The lack of hypotensive action of acupuncture in sham-operated control animals remains an unexplained but reproducible observation.

Herbal medicine and hypertension

Herbal medicine has been used in Asia for a long time to treat hypertension. Chinese herbs, such as *Ramulus cum Uncis Uncariae* ("Gou teng" in Chinese), *Radix Salviae Miltiorrhizae* ("Dan shen"), *Herba Siegesbeckiae* ("Xi xian cao"), *Cortex Moris Albae Radicis* ("Sang bai pi"), *Spica Prunellae Vulgaris* ("Xia ku cao") and *Folium Clerodendri Trichotomi* ("Chou wu tong"), have been used traditionally for the treatment of hypertension [40].

Oral administration of *Folium Clerodendri Trichotomi* reduces blood pressure of spontaneously hypertensive rats, but not for normotensive control rats [41]. Chronic daily administration of the herb for 6 weeks prevented the increase in blood pressure of spontaneously hypertensive rats [41]. Mixtures of Chinese herbs are also effective in reducing blood pressure in mild hypertension [42]. *Herba Siegesbeckiae* and *Cortex Moris Albae Radicis* exhibited the property to lower blood pressure in anesthetized animals [40].

Among the Chinese herbs, *Ramulus cum Uncis Uncariae* has been used empirically for a long time in the treatment of hypertension. Recently, evidence of the antihypertensive and vasodilating effects of *Ramulus cum Uncis Uncariae* has been reported in several studies [43-45]. These findings suggest that *Ramulus cum Uncis Uncariae* contains active substances with vasodilating or vasorelaxant effects. This action may be related to its ability to block α_1-adrenoreceptors and Ca^{2+} channels [46, 47]. Further, *Ramulus cum Uncis Uncariae* may produce relaxation through the endothelium-dependent relaxing factor, now known as nitric oxide (EDRF/NO), in isolated rat aorta [45]. To date, various indole alkaloids have been isolated and identified from *Ramulus cum Uncis Uncariae* [47-49]. The major indole alkaloids are hirsutine, hirsuteine, rhynchophylline, isorhynchophylline, corynoxeine, and isocorynoxeine. Among the indole alkaloids, the vasorelaxant activity of hirsutine especially has been demonstrated to be more potent than those of other products such as rhynchophylline and isorhynchophylline [47, 50]. The lethal dose 50 (LD_{50}) in mice for one dose of a decoction of *Ramulus cum Uncis Uncariae* is 29 g/kg [40].

Radix Salvia miltiorrhizae is another Chinese herb that ha been used traditionally in many Chinese medicine preparations and formulae. Radix Salvia miltiorrhizae is believed to be effective in eliminating blood stasis, relieving pain, promoting blood flow, stimulating menstrual discharge and easing the mind

[40]. Recent pharmacological studies have indicated that both aqueous and lipid soluble fractions of *Radix Salvia miltiorrhizae* contain the active components responsible for some of the observed clinical effects. The two active hydrophilic components of *Radix Salvia miltiorrhizae* are "Danshensu" and magnesium tanshinoate B (MTB, also named lithospermic B magnesium salt, or magnesium lithospermate B), while cryptotanshinone and tanshinone II_A are the two lipophilic components [51]. These four components are responsible for many of *Radix Salvia miltiorrhizae*'s actions. Among the hydrophilic active components, MTB has strong antioxidative and free radical scavenging effects [52-54], and stimulates the release of NO from endothelial cells [55]. MTB protects against systemic pathologic processes such as renal dysfunction, liver damage, and ischemia-reperfusion injury [54, 56, 57]. Among the lipophilic components, tanshinone II_A has antioxidant properties and protects against lipid peroxidation in vitro and in vivo, making it a potential antidote for free radical based disorders [58-60]. Tanshinone II_A has neuroprotective effects in cerebral ischemia and attenuates hypertrophy induced by angiotensin II [61, 62]. There is great interest in the therapeutic potential of MTB and tanshinone II_A. Unfortunately, little attention has been paid to the mechanisms of action of MTB and tanshinone II_A on hypertension. We will address possible mechanisms of action of *Radix Salvia miltiorrhizae* and its active component tanshinone II_A on hypertension *in vivo*. The toxicity level of the LD_{50} for peritoneal injection of Radix Salviae miltiorrhizae in mice is 36.7g/kg [40].

Ramulus cum Uncis Uncariae (Uncariae) and Radix Salvia miltiorrhizae significantly reduce blood pressure in hamsters

To develop a new animal model for research into the mechanisms of the beneficial actions of herbal medicine in hypertension, we studied the impact of the administration of the Chinese herbs *Uncariae* and *Radix Salvia miltiorrhizae* in renovascular hypertensive hamsters (2K1C and their sham-operated controls).

Studies with Ramulus cum Uncis Uncariae (Uncariae)

We purchased Uncariae from Ming Tong Pharmaceutical Company, Taiwan. Hamsters in the experimental groups received Uncariae once a day by oral gavage (0.5 mL saline solution) starting two weeks after the 2K1C surgical operation. The treatment was continued for two weeks. Non-treated groups underwent the exact same procedure as the treatment groups, except without *Uncariae*.

MAP in the control, sham-operated normotensive hamsters was 117.0±6.9 mmHg. Renovascular hypertension (2K1C) was demonstrated by elevation of blood pressure to 164.0±6.6 mmHg. *Uncariae* produced a dose-related blood pressure reduction in 2K1C hamsters. At 30 mg/100g body-weight (bw), *Uncariae* significantly reduced MAP to 138.1±1.7 mmHg; whereas at a dose of 60 mg/100g.bw *Uncariae* reduced MAP to 105.2±10.0 mmHg ($p<0.05$ compared to 2K1C MAP). These results are shown on **Fig. 2**.

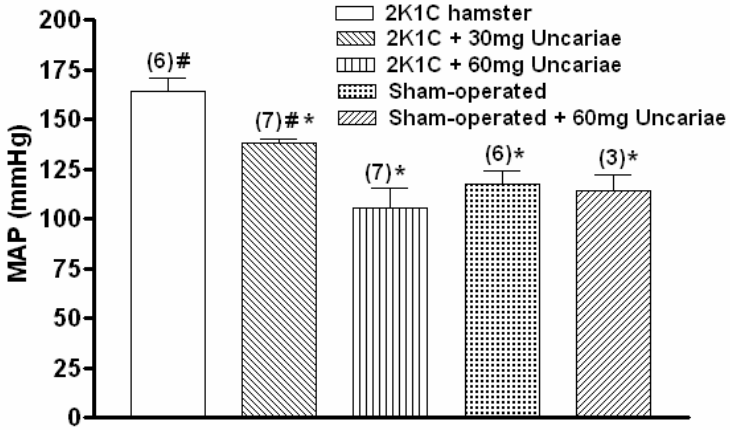

Fig. 2 Effect of *Ramulus cum Uncis Uncariae* on mean arterial blood pressure after induction of hypertension. The bars represent the mean SEM. The numbers in parenthesis show the number of hamsters included in each group. *$p<0.05$ compared with non-treated 2K1C hamsters. $^{\#}p<0.05$ compared with sham-operated hamsters.

To investigate the effect of *Uncariae* on a normotensive hamster, we applied 60 mg *Uncariae* on sham-operated hamster group. There was no significant mean arterial blood pressure difference between sham-operated hamsters with *Uncariae* treatment and those without *Uncariae* treatment.

Studies with Radix Salviae miltiorrhizae (RSM)

We used 2K1C renovascular hypertensive hamsters to test the hypothesis that RSM (purchased from Ming Tong Pharmaceutical Company, Taiwan) has anti-hypertensive effects. We administered a dose of 40 mg/100g of body weight by oral gavage (0.5 mL of saline solution) once a day for 2 weeks prior to experimental measurements.

Normotensive sham-operated control hamsters had a MAP of 115.0±7.2 mmHg. Renovascular hypertension was demonstrated in 2K1C hamsters by elevation of MAP to 160.0±7.6 mmHg. After 2-week of herbal treatment, RSM (40 mg/100 g.bw) significantly reduced MAP to 121.1±6.9 mmHg ($p<0.05$ *versus* MAP of 2K1C hamsters); indicating that RSM inhibited or rescued the hypertension induced by the 2K1C surgery. RSM at 40 mg in the sham-operated hamsters showed a tendency towards hypotension compared with non-treated sham-operated hamsters, but the reduction in MAP was not significantly different. The results are displayed in **Fig. 3**.

We took advantage of the commercial availability of Tanshinone II_A (Ningbo Shuanglin Traditional Chinese Medicine Co, Ningbo, China), one of the active lipophilic components of *Radix Salvia miltiorrhizae* in order to refine the experimental assessments of our hypothesis. Tanshinone II_A (chemical formula = $C_{19}O_3H_{20}$) is a derivative of phenanthrenequinone. The purity of tanshinone II_A was greater than 95 %. Experimental constriction of the renal artery increased MAP to 161.2±6.9 mmHg (mean SEM) relative to 114.3±9.2 mmHg in age-matched non-treated normotensive sham operated control hamsters. Treatment with 50 g tanshinone II_A/ 100g.bw for two weeks reduced MAP from 161.2±6.9 to 130.0±7.8 mmHg (**Fig. 4**). Tanshinone II_A had no significant influence on mean arterial blood pressure in normotensive hamsters (112.1±8.3 mmHg *vs.* 114.3±9.2 mmHg treated *vs* non-treated hamsters, respectively). Administration of 0.2% dimethyl sulfoxide (DMSO), the solvent for tanshinone II_A, did not change MAP of 2K1C hypertensive hamsters relative to hypertensive hamsters that did not receive DMSO (160.0±7.6 mmHg *vs.* 161.2±6.9 mmHg, respectively).

Hypertension and microcirculation

In most forms of clinical and experimental hypertension, blood pressure is elevated in proportion to the increase in peripheral vascular resistance. Pressure profile analyses demonstrate that the microcirculation is the major site of vascular resistance [63].

An increase in the wall-to-lumen ratio of small arteries and arterioles is an important mechanism that causes an increase in resistance and leads to hypertension [64, 65]. The underlying assumption is that wall hypertrophy produces lumen encroachment

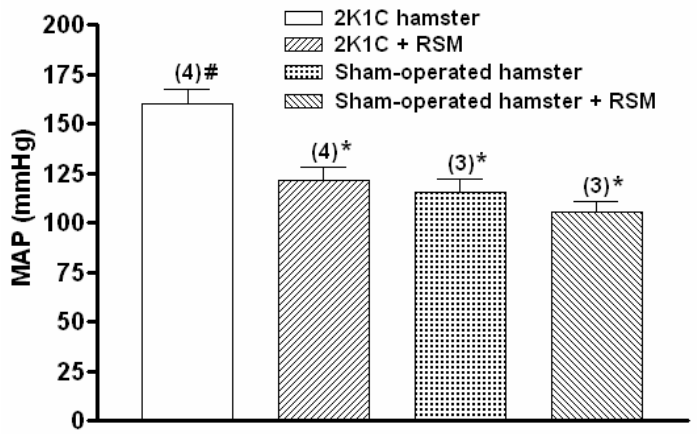

Fig. 3 Effect of Radix Salviae miltiorrhizae (RSM) on mean arterial blood pressure (MAP) after induction of hypertension. Bars represent mean SEM. Numbers in parenthesis show the number of hamsters included in each group. *$p<0.05$ compared with non-treated 2K1C hamsters, #$p<0.05$ compared with sham-operated hamsters.

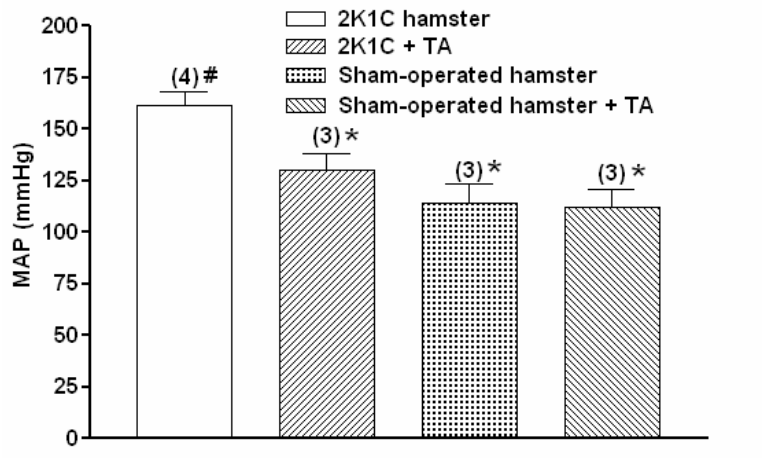

Fig. 4 Tanshinone II_A reduces mean arterial blood pressure (MAP) in experimental renovascular hypertension. The bars represent mean SEM. The number in parenthesis show the number of hamsters included in each group. TA=tanshinone II_A, *$p<0.05$ compared with non-treated renovascular hypertensive (2K1C) hamsters, #$p<0.05$ compared with sham-operated hamsters. (Modified from [36]; reproduced with permission).

under resting conditions and reduces the available vessel radius. Evidence of wall hypertrophy, either by cellular hypertrophy or cellular hyperplasia, has been reported in virtually every form of hypertension [66, 67].

Another factor that influences vascular resistance is the number of microvessels per unit mass of tissue. The phenomenon of the reduced number of microvessels per unit tissue mass is termed "rarefaction". Experimental evidence indicates that microvascular rarefaction is an important pathogenic mechanism in several models of hypertension [68-70] including human hypertension [65, 71]. This microvascular alteration may cause a significant increase in peripheral vascular resistance and contribute to elevated blood pressure.

The regulation of blood pressure is based on counter-balancing vasoconstrictor and vasodilator systems. Angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II. Angiotensin I, produced mainly in the kidneys, is converted to angiotensin II by angiotensin-converting enzyme (ACE). Because angiotensin II is a powerful vasoconstrictor in the microcirculation, reduction of its plasma levels lowers arterial blood pressure. ACE activity is influenced by nitric oxide (NO) as indicated by the up-regula-tion of cardiac and vascular ACE activity induced by chronic inhibition of eNOS [72]. In contrast, stimulated endothelial NO release as well as NO released from donor compounds can inhibit ACE activity and reduce the conversion of angiotensin I to angiotensin II [73]. Conversely, long-term inhibition of vascular and cardiac ACE expression and activity causes up-regulation of eNOS expression and increased vascular NO release in hypertensive rats [74], and prevents arterial hypertension induced by chronic NO synthesis inhibition [75]. Taken together, these observations suggest a 'cross talk' between eNOS expression/activity and tissue ACE expression/activity by means of feedback regulation.

Elevation in plasma renin activity is one of the characteristics of renovascular hypertension [38, 39, 76]. Measurements in the laboratory showed that control and sham-operated hamsters have similar levels of plasma renin activity; 11.6±1.0 ng-Angiotensin I(AI)/ml/hour for na ve control; and 10.1±1.2 ngAI/ml/hour for sham-operated animals (mean±SEM), while two-kidney, one-clip renal hypertensive (2K1C) hamsters have a significantly higher value two weeks after 2K1C surgery (33.4±8.8 ngAI/ml/hour; $p<0.05$ *vs*. both control and sham-operated) [38]. The difference in plasma renin activity became insignificant after 3 weeks [38]. The increment in plasma renin activity was associated with a significant and chronic elevation in blood pressure in 2K1C hamsters from 112.3±6.2 (control) to 160.1±7.5 mmHg [3-days post-surgery]; 160.0±5.5 mmHg [6-weeks post-surgery]; $p<0.05$ compared to control) [38].

Endothelial nitric oxide synthase signaling mechanisms in hypertension

Endothelial nitric oxide synthase (eNOS) is an important regulator of vascular homeostasis because it is the major source of nitric oxide (NO) production

in vascular endothelial cells. eNOS plays a crucial role in determining microvascular diameter and hence peripheral resistance and blood pressure [77, 78]. Further, NO released by the endothelium modulates other processes including hyperpermeability, platelet aggregation, platelet and leukocyte adhesion to the endothelium, vascular smooth muscle cell proliferation, and angiogenesis [79-83]. Signaling interactions between the vascular wall and blood cells provide a unique way of communicating, coordinating and integrating an appropriate physiologic response to the regulation of blood pressure. The loss or attenuation of bioavailable NO production in endothelium is one of the earliest biochemical markers of endothelial dysfunction found in many cardiovascular diseases including hypertension [77, 78, 84, 85].

Nitric oxide derived from eNOS is a major regulator of vessel remodeling in response to stimuli. Luminal remodeling is impaired in the absence of eNOS, so that the vessel wall thickness doubles due to proliferation of vascular smooth muscle cells [86, 87]. These studies support the concept that NO is a major determinant of vessel architecture in response to hemodynamic stimulation. NO may however not be the principal factor in rarefaction inasmuch as arteriolar rarefaction occurs in eNOS knockout mice; an observation indicating that rarefaction develops mainly in association with the rise in blood pressure, and does not depend on the loss of eNOS as the main source of nitric oxide production [88].

eNOS-derived NO is an endogenous gas that regulates the diameter of blood vessels and maintains an anti-proliferative and anti-apoptotic environment in the vessel wall. Initially thought to be a simple, calmodulin-regulated enzyme, eNOS has evolved as an enzyme that is tightly controlled by co- and post-translational lipid modifications, phosphorylation on multiple residues and regulated protein-protein interactions and subcellular translocation [89, 90]. Elegant studies have demonstrated that Akt (also known as protein kinase B or PKB) phosphorylates eNOS at Ser-1179, and leads to NO production *in vitro* [91] and in vivo [92]. In addition, eNOS dephosphorylation at specific sites is associated with the synthesis of NO [93, 94].

The significance of eNOS in hypertension is demonstrated by the development of elevated blood pressure in eNOS knockout (KO) mice. Interestingly, hypertension develops in eNOS-KO mice as a function of time. The difference in mean arterial pressure between eNOS KO and eNOS wild-type mice becomes significant at 12-weeks of age [69, 88]. The lack of the eNOS gene in the eNOS-KO animals shows the importance of eNOS activity in the development of hypertension.

The short-term and long-term administration of N^{W}-nitro-L-arginine methyl ester (L-NAME) to inhibit NOS increases arterial blood pressure [95-97]. The fact that NOS-inhibited animals become hypertensive further supports the concept that NO is an important element in the regulation of microvascular diameter and blood pressure. Chronic administration of L-NAME also leads to functional and morphological alterations in the vascular endothelium and vascular smooth muscle cells in hypertension [98]. In fact, chronic administration of L-NAME significantly decreases the levels of aortic eNOS mRNA compared to those of control rats [99]. Consistent with the experimental evidence, newborn humans who present with persistent pulmonary hypertension exhibit decreased eNOS gene expression in their umbilical vein endothelial cells [100].

Acupuncture and herbal medicine either prevent or rescue functional microvascular alterations in hypertension

Based on the above described human and experimental data, it was hypothesized that the level of eNOS activity/expression is impaired in experimentally induced renovascular hypertension, and acupuncture and herbal treatment will prevent or improve the impaired eNOS activity/expression. This hypothesis was tested in two-kidney, one-clip renal hypertensive (2K1C) hamsters and their appropriate controls.

As a first approach, it was investigated whether or not acupuncture and herbal medicine would influence the relationship between arteriolar wall thickness (W) and luminal diameter (L). **Table 1** shows W/L ratios in non-treated two-kidney, one-clip renal hypertensive (2K1C) hamsters following renal artery clipping, sham-operated hamsters, and five treatment groups after two weeks of acupuncture and/or herbal treatments. To quantify wall-to-lumen ratio, computer-assisted image analysis was used to measure the luminal diameter and wall thickness from a replay of the images captured with brightfield microscopy from the hamster cheek pouch. The average wall-to-lumen ratio was calculated from two or three fields of 2^{nd} and 3^{rd} order arterioles per

animal. The wall-to-lumen ratios in non-treated 2K1C hamsters were significantly higher than in sham-operated hamsters. Acupuncture on Shixuan point induced a significant improvement in wall-to-lumen ratio. Herbal treatment with *Uncariae* (once a day by oral gavage) for two weeks also significantly reduced wall-to-lumen ratios compared with those of non-treated 2K1C hamsters. A combined treatment of herbal medicine and acupuncture on Shixuan point was as effective as *Uncariae* alone in either preventing or reducing the wall-to-lumen ratio increments in renovascular hypertensive hamsters.

Table 1 displays the association between renovascular hypertension, complementary and alternative medicine treatments and arteriolar density (which is an inverse index of rarefaction). Quantitative stereological techniques and computer-assisted digital image processing were used to determine arteriolar density, which is expressed as vessel length per unit area of tissue [101]. The analysis was based on data obtained using intravital fluorescent microscopy, in which fluorescein isothiocyanate-dextran 70 (FITC-Dx 70) served better to delineate the lumen of the microvessels. The development of renovascular hypertension was associated with rarefaction as shown by the significant decrease in arteriolar density at 4 weeks after surgery in the 2K1C groups. Acupuncture on Shixuan point showed a positive trend but per se did not significantly reduce rarefaction in 2K1C hamsters. Herbal treatment with Uncariae was efficacious in significantly preventing or reducing arteriolar rarefaction in the 2K1C hamsters. Arteriolar density in 2K1C-Uncaria treated group was comparable to those observed in the control groups. The combination of acupuncture and herbal treatment is as effective as herbal treatment alone. The data strongly demonstrate that treatment with herbs was effective and efficacious in either preventing rarefaction or restoring arteriolar density to normal values (**Table 1**).

Endothelial nitric oxide as a target for acupuncture and Chinese herbs in hypertension

It was postulated that reduction in blood pressure in renovascular hypertensive animals is mediated by changes in expression or activity of eNOS. Due to our experience with microvascular studies in the hamster cheek pouch, we tested the impact of acupuncture on the stomach 36 (ST-36) acupoint. The choice of ST-36 (located on the stomach meridian, which includes the cheek pouch) allows for acupuncture stimulation at a site remote from the microvascular test site.

Acupuncture on ST-36 increases periarteriolar NO concentration in hamster cheek pouch microcirculation

To test our hypothesis, we investigated the impact of acupuncture on the production of microvascular NO in normotensive and renovascular hypertensive hamsters. We measured periarteriolar NO concentration with NO-sensitive microelectrodes [24, 36, 102].

The mean periarteriolar concentration of NO was 435.1±25.7 nM in arterioles with mean diameter of 38 3 m in sham operated hamsters. Renovascular hypertension reduced mean periarteriolar NO concentration to 309.0±21.7 nM in 2K1C hamsters ($p<0.05$) as shown in **Fig. 5**. Acupuncture-treated 2K1C hamsters showed periarteriolar NO concentrations of 417.9±20.9 nM, which were comparable to those of normotensive hamsters and represent a significant increase relative to non-treated 2K1C renovascular hypertensive hamsters. These data support the concept that electroacupuncture on ST-36 can restore the bioavailability of NO and perivascular NO concentration in the arterioles of hypertensive animals.

Table 1. Wall-to-lumen ratio (W/L) and arteriolar density (AD: mm/mm^2) in the hamster cheek pouch. The data represent mean±SEM. The numbers in parentheses show the number of animals included in each group. Acu=Acupuncture treatment on Shixuan point. *$p<0.05$ compared with sham-operated hamsters. $^{\#}p<0.05$ compared with 2K1C without treatments.

2K1C (6)		**Sham-operated (6)**	**Acupuncture (6)**	
W/L	0.191±0.002*	0.132±0.001	0.172±0.002*$^{\#}$	
AD	2.5±0.2*	3.3±0.2	2.8±0.1*	
	30 mg Uncariae (7)	**60 mg Uncariae (7)**	**Acu+30 mg Uncariae (7)**	**Acu+60 mg Uncariae (7)**
W/L	0.153±0.004*$^{\#}$	0.139±0.007#	0.152±0.006*#	0.134±0.004$^{\#}$
AD	3.0±0.2$^{\#}$	3.3±0.1$^{\#}$	3.1±0.1$^{\#}$	3.3±0.2$^{\#}$

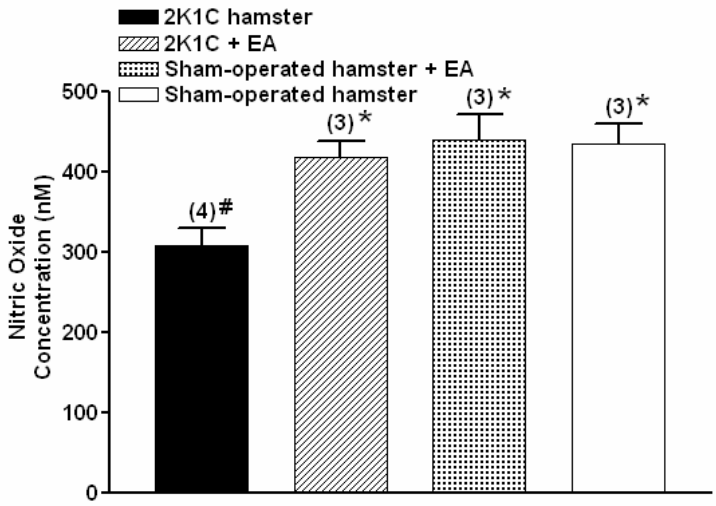

Fig. 5 Electroacupuncture on ST-36 increases periarteriolar NO concentration. The bar graph shows the maximal increments in response to treatment. The data represent the mean±SEM. The numbers in parenthesis show the number of animals included in each group. EA=electroacupuncture, *p<0.05 compared with non-treated 2K1C hamsters, #p<0.05 compared with sham-operated hamsters. (Modified from [24]; reproduced with permission).

To determine whether the benefits of increased periarteriolar NO concentration were associated with changes in the expression of eNOS, we measured eNOS protein using western blotting. To standardize the analysis, the results were expressed as the ratio of the intensity of the experimental to the corresponding sham-operated control group (i.e., 2K1C/sham). The results of the net band intensities ratios yielded values of 1.06±0.09 (treated sham-operated group), 0.85±0.04 (2K1C hypertensive group), and 1.21±0.06 (treated 2K1C group) relative to the non-treated sham-operated control group in the hamster cheek pouch (**Fig. 6**). These data clearly demonstrate that 1) renovascular hypertension reduces the amount of eNOS in the microvasculature, and 2) electroacupuncture on ST-36 restores the mass of eNOS to normal values. Our results also support the concept that damaging alterations in eNOS (expression or activity) contribute to the development of hypertension.

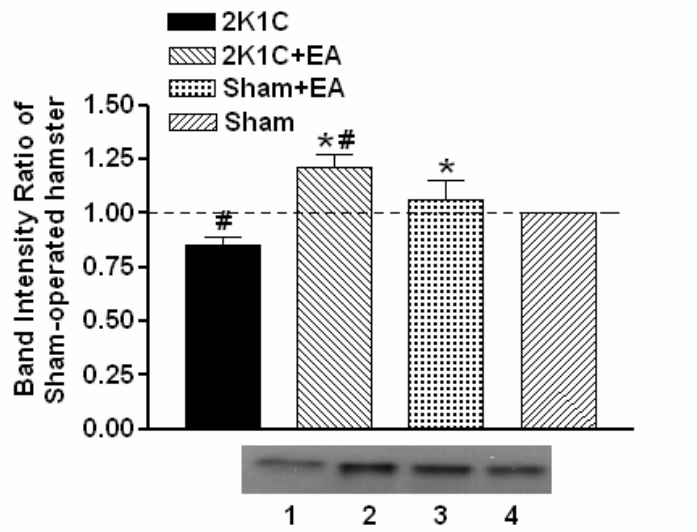

Fig. 6 Western blotting analysis of eNOS in hamster cheek pouch. The band intensities in the Western blots were divided by the band intensity of corresponding non-treated sham-operated hamster. The data represent the mean±SEM. Each group represents three hamsters. EA: electroacupuncture, *P<0.05 compared with non-treated 2K1C hamsters, #p<0.05 compared with sham-operated hamsters. The lower panel shows representative western blot bands for each group. **1**: 2K1C, **2**: 2K1C+EA, **3**: Sham-operated+EA, **4**: Sham-operated. (Modified from [24]; reproduced with permission).

Radix Salviae miltiorrhizae and tanshinone II_A increase NO production in the microcirculation

We tested the hypothesis that nitric oxide synthesis is involved in the biochemical-signaling pathway of Radix Salviae miltiorrhizae. A consequence of NO production in arterioles is vasodilation. For this reason, as a first approach, changes were measured in vessel diameter (which is expressed as the ratio of the experimental to the control diameter, with control diameter normalized to 1), using bright-field trans-illumination microscopy and computer-assisted digital image analysis. NG-Monomethyl-L-Arginine (L-NMMA) was used at 10^{-5} M to inhibit NO synthase. This concentration of L-NMMA effectively blocks acetylcholine and bradykinin induced vasodilation [103]. *Radix Salviae miltiorrhizae* at 2mg/ml significantly increased arteriolar diameter to 1.38±0.17 ($p<0.05$; **Fig. 7**). L-NMMA at 10^{-5}M efficaciously attenuated increase. These results support the hypothesis that nitric oxide production is a step in the biochemical-signaling pathway of the microvascular vasodilation responses to *Radix Salviae miltiorrhizae*.

If nitric oxide synthase plays a role as a signaling molecule, then the agonist must stimulate the production of NO. To test this required step of the hypothesis, 5 μg/mL tanshinone II_A was applied topically, and measured periarteriolar NO concentration with NO-sensitive microelectrodes. The mean control diameter of the selected test arterioles was 40±2 μm. **Figure 8** shows that topical application of 5 μg/mL tanshinone II_A significantly increased periarteriolar NO concentration from 87.1±11.3 nM to 146.9±23.1 nM ($p<0.05$). The periarteriolar NO concentration reached its maximum value during the 5 minute topical application and declined slowly after removal of tanshinone from the suffusate; baseline levels were achieved about 15-20 minutes later. The vehicle did not change periarteriolar NO concentration.

Tanshinone II_A increases eNOS protein in the hamster cheek pouch

Having demonstrated that tanshinone reduces systemic arterial blood pressure and is able to increase NO production, we tested the hypothesis that these beneficial effects are due to up-regulation of eNOS. The expression of eNOS protein in hamster cheek pouch was determined by Western blotting. The results were expressed as the ratio of the intensity of the experimental to the corresponding sham-operated control group in order to standardize the analysis, (*i.e.*, 2K1C/sham). The analysis of the net band intensities yielded ratios of 0.79±0.03 (2K1C hypertensive group), 1.15±0.05 (treated 2K1C group), and 1.01±0.07 (treated sham-operated group) (**Fig. 9**). The results show that a) hypertension reduced the expression of eNOS relative to the sham-operated control group ($p<0.05$); b) tanshinone prevented the reduction of eNOS associated with hypertension, and showed even higher eNOS protein expression than the sham-operated control in hamster cheek pouch ($p<0.05$).

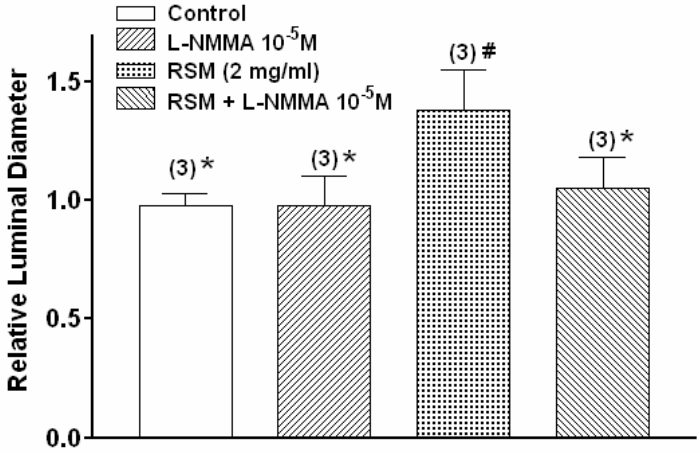

Fig. 7 Interactions between inhibition of NOS and *Radix Salviae miltiorrhizae*-induced vasodilation. Bars represent the mean±SEM. Numbers in parenthesis show the number of animals included in each group. RSM= *Radix Salviae miltiorrhizae*, *$p<0.05$ compared with RSM, #$p<0.05$ compared with control.

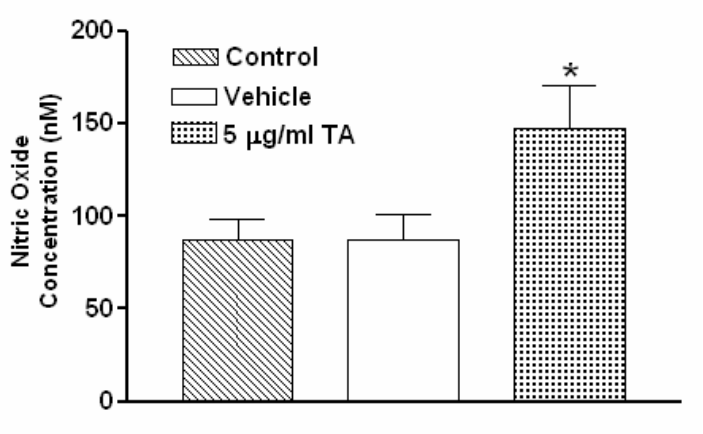

Fig. 8 Tanshinone II_A increases periarteriolar NO concentration. Bar graph shows maximal increments in response to tanshinone II_A (TA) in hamster cheek pouch. Data represent mean±SEM. Each group represents three hamsters. *p<0.05 compared with control. (Modified from [36]; reproduced with permission).

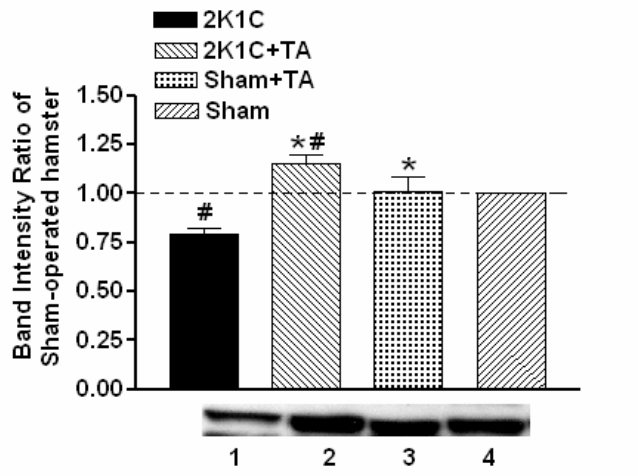

Fig. 9 Western blotting analysis of eNOS in hamster cheek pouch. The band intensities in the Western blots were divided by the band intensity of corresponding non-treated sham-operated hamster. Data represent mean±SEM. Each group represents three hamsters. TA=tanshinone II_A, *p<0.05 compared with non-treated 2K1C hamsters, #p<0.05 compared with sham-operated hamsters. The lower panel shows representative Western blot bands for each group. **1**: 2K1C, **2**: 2K1C+TA, **3**: Sham-operated +TA, **4**: Sham-operated. (Modified from [36]; reproduced with permission).

The meridian theory and therapeutic mechanisms for hypertension

In order to better understand the connections between Western and traditional Oriental Medicine, the relationship between the point of acupuncture treatment and the underlying biochemical mechanism was explored. Because the biologic mechanisms of the meridian system and transduction of acupuncture signals on the meridian system remain unknown, this task was approached by testing the applicability of the meridian theory to our results. First, we present a global view of our understanding of the meridian theory, and then we present our experimental results.

The meridian theory is an essential pathway system, described and applied in acupuncture and oriental medicine for thousands of years, which deals with physiological regulation and pathological changes of the human body [4, 104, 105]. It is recorded in the *Yellow Emperor's Classic of Medicine*,one of the earliest medical classics in China that "the meridians are the place where life and death are determined, disease is generated, treated and cared for; they are the place where acupuncture starts and acupuncture ends" [106]. This demonstrates the great importance of the meridians in physiology, pathology, diagnosis and treatment in acupuncture and oriental medicine.

Thus, in clinical practice, the meridian system serves as a guideline in differential diagnosis and as a basis to select the acupoints along the meridians for treatment.

According to acupuncture and oriental medicine, a form of bodily energy is generated in the internal organs and systems [4, 105]. This energy combines with breath and circulates throughout the body, forming paths called meridians. The meridians form a complex, multilevel network which connects the various areas of the body. All the various meridian systems work together to assure the flow and distribution of energy throughout the body, thereby controlling all bodily functions. When an organ or a system does not have a balanced energy, acupuncture points along the meridians are stimulated, affecting the circulation of energy, which in turn affects the related internal organ and system to make a balance of energy [4, 105].

Acupuncture at specific acupoints stimulates organs in defined meridians

The results discussed earlier demonstrated that acupuncture at ST-36 stimulates an increase in eNOS protein in the hamster cheek pouch, which is located in the stomach meridian. The stomach meridian starts at the lateral side of the nose, ascends laterally along the infra-orbital ridge, and continues laterally across the cheeks. It passes through the diaphragm, enters the stomach, and connects with the spleen and the heart, specifically through the stomach divergent channel. Next, it descends inside the abdomen, reaches the knee, and ends at the lateral side of the tip of the 2nd toe [4, 105]. To further assess that the stomach is the central organ in the stomach meridian, and is influenced by acupuncture, eNOS protein was measured using Western blotting, in samples from the stomachs of hamsters undergoing renovascular hypertension and acupuncture on ST-36, using the same four experimental groups.

eNOS was detected in the stomach in all groups, and their respective band intensities was measured. The respective values in the hamster stomach were 0.77±0.04 (2K1C hypertensive group), 1.05±0.15 (treated sham-operated group), and 1.08±0.07 (treated 2K1C group) relative to the non-treated sham-operated control group in the hamster stomach (**Fig. 10**). Hypertension reduced the expression of eNOS relative to the sham-operated group. Acupuncture treatment on ST-36 either prevented or rescued the reduction of eNOS associated with hypertension in the hamster stomach. Because the stomach and the cheek pouch are organs on the stomach meridian, these data support the concept that the stimulation of an increase in eNOS proceeds along the meridian connected to the stimulated acupoint.

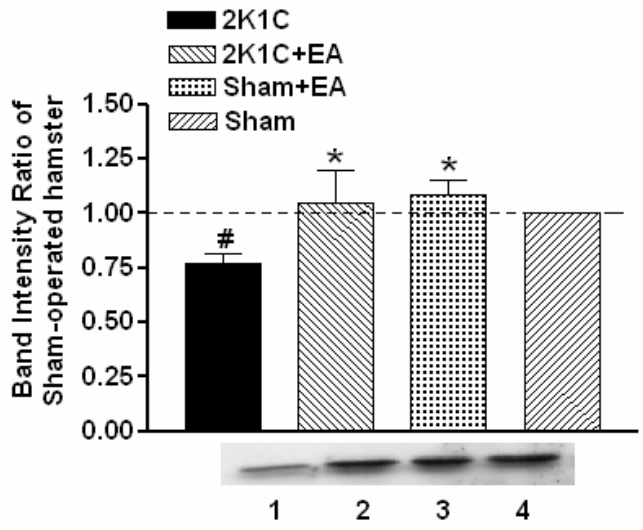

Fig. 10 Western blotting analysis of eNOS in stomach. The band intensities in the Western blots were divided by the band intensity of corresponding non-treated sham-operated hamster. The data represent the mean±SEM. Each group represents three hamsters. EA: electroacupuncture, *p<0.05 compared with non-treated 2K1C hamsters, #p<0.05 compared with sham-operated hamsters. The lower panel shows representative western blot bands for each group. **1**: 2K1C, **2**: 2K1C+EA, **3**: Sham-operated+EA, **4**: Sham-operated. (Modified from [24]; reproduced with permission).

To further test the meridian theory, we examined the expression of eNOS protein in liver, an organ that is not on the stomach meridian, by Western blotting. **Figure 11** shows the analysis of the net band intensities in liver. The ratios (relative to non-treated sham-operated control group) were 0.82±0.04 (2K1C group), 0.85±0.04 (treated 2K1C group), and 1.01±0.05 (treated sham-operated group). Analysis of liver tissue indicated that electroacupuncture on ST-36 did not have a significant benefit in terms of enhanced expression of eNOS in the treated 2K1C hypertensive group relative to the non-treated 2K1C hypertensive group. The liver eNOS levels in non-treated 2K1C hamsters were significantly reduced relative to sham-operated hamsters.

In support of the meridian theory, our results demonstrate that stimulation of ST-36 activates specific targets on the stomach meridian (cheek pouch and stomach), but does not activate them in organs located on separate meridians (liver). Based on this ancient stomach meridian theory, the meridian passes through not only the stomach, but also the spleen and the heart. Whether these organs are involved for the reduction of blood pressure by ST-36 acupuncture treatment needs further investigation.

General conclusions

Acupuncture and herbal medicine efficiently and efficaciously did reduce elevated blood pressure in animal models of human hypertension. Our studies demonstrated in hamsters that acupuncture on stomach 36 point reduces experimental renovascular hypertension and increases the production of NO in arterioles. Importantly, our studies are consistent with the theory that acupuncture on ST-36 proceeds along the stomach meridian as indicated by the up-regulation of eNOS in organs located in the stimulated meridian (such as stomach and cheek pouch), but not in organs located in the liver meridian (liver).

The evidence reviewed here provides a basis for better understanding the microvascular mechanisms of the action of acupuncture and of herbal medicine as used in the treatment of hypertension in the practice of complementary and alternative medicine. In this context, it was concluded that activation of eNOS is one of the mechanisms through which acupuncture and Chinese herbs reduce blood pressure. We also conclude that ST-36 acupuncture-induced reduction of blood pressure proceeds and works through the stomach meridian.

In addition, it was concluded that enhanced microvascular eNOS protein synthesis, and its associated increase in NO production are important mechanisms by which the active ingredients of Chinese herbs - such as tanshinone II_A, an active lipophilic ingredient of *Radix Salviae miltiorrhizae*-decrease blood pressure in hypertension.

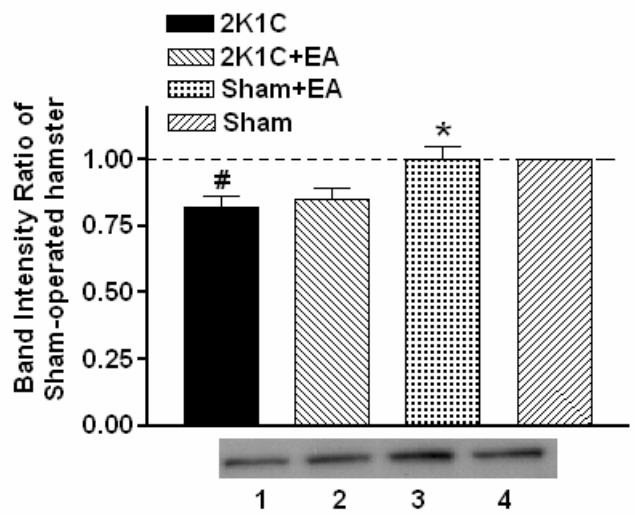

Fig. 11 Western blotting analysis of eNOS in liver. The band intensities from the Western blots were divided by the band intensity of corresponding non-treated sham-operated hamster. The data represent the mean±SEM. Each group represents three hamsters. EA=electroacupuncture, *$p<0.05$ compared with non-treated 2K1C hamsters, $^{\#}p<0.05$ compared with sham-operated hamsters. The lower panel shows representative western blot bands for each group. **1**: 2K1C, **2**: 2K1C+EA, **3**: Sham-operated+EA, **4**: Sham-operated. (Modified from [24]; reproduced with permission).

A better understanding of the microvascular mechanisms of action of acupuncture and herbal medicine used in the treatment of hypertension should contribute to bring closer together the practices of complementary and alternative medicine and allopathic medicine in the treatment of hypertensive patients.

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