Common parameters of acupuncture for the treatment of hypertension used in animal models

Lu Shengfeng, Cao Xin, Hiroshi Ohara, Yuji Nakamura, Hiroko Izumi-Nakaseko, Kentaro Ando, Liu Wanxin, Atsushi Sugiyama, Zhu Bingmei

Lu Shengfeng, Liu Wanxin, Zhu Bingmei, Key Laboratory of Acupuncture and Medicine Research of Ministry of Education, Nanjing University of Chinese Medicine, Nanjing 210023, China
Cao Xin, Hiroshi Ohara, Yuji Nakamura, Hiroko Izumi-Nakaseko, Kentaro Ando, Liu Wanxin, Atsushi Sugiyama, Department of Pharmacology, Faculty of Medicine, Toho University, Tokyo 143-8540, Japan
Supported by the National Basic Research Program of China: the Epigenetic Regulatory Mechanism of the Specificity of Acupoints (973 Program, No. 2012CB518501); the National Natural Science Foundation of China: the Regulatory Mechanism of AS-Neuron Crosstalk With Energy for Promoting Synaptic Plasticity in Hippocampus Treated by Acupuncture (No. 81202743); Deacetylation Modification in Personalized Medicine for Acupuncture-Based Weight-Loss (No. 81273838); Mechanistic Study of STAT5-miR-193b Pathway in Obesity and Weight Loss by Acupuncture (No. 81273838)
Correspondence to: Prof. Zhu Bingmei, Key Laboratory of Acupuncture and Medicine Research of Ministry of Education, Nanjing University of Chinese Medicine, Nanjing 210023, China; Prof. Atsushi Sugiyama, Department of Pharmacology, Toho University, Tokyo 143-8540, Japan. zhubm64@hotmail.com; atsushi.sugiyama@med.toho-u.ac.jp
Telephone: +86-25-85811235; +86-15050583071
Accepted: June 28, 2014

Abstract
Hypertension is associated with at least 7.6 million annual deaths worldwide. While pharmacotherapy may provide good control for blood pressure, it sometimes induces adverse effects. Meanwhile, acupuncture has been used for the treatment of cardiovascular diseases, such as hypertension, coronary artery disease, and stroke, but its mechanisms of actions remain poorly understood. The efficacy of acupuncture depends on multiple constituent elements including acupoints, manipulation skills, and implementation programs, which are termed as acupuncture prescription. This review summarized the previous information of experimental use of acupuncture on animals including species, hypertension models, acupoints selection, acupoint location, stimulation protocols, and evaluation of effectiveness to provide useful guidance for researchers when performing acupuncture in animal experiments.

© 2015 JTCM. All rights reserved.

Key words: Acupuncture; Animals; Cardiovascular diseases; Hypertension; Drug therapy; Review

INTRODUCTION
Hypertension, affecting approximately 1 billion individuals worldwide, is a major risk factor for cardiovascular morbidity and mortality, which is associated with at least 7.6 million annual deaths worldwide (13.5% of all deaths), as well as a significant increase in healthcare cost. Hypertension models, acupoints selection, acupoint location, stimulation protocols, and evaluation of effectiveness to provide useful guidance for researchers when performing acupuncture in animal experiments.
Acupuncture is a traditional modality of treatment that consists of four elements based on the theory of ancient Chinese medicine, including pattern identification, needling technique, acupoints selection, and stimulation parameters. Although acupuncture has historically been used to treat hypertension in China, its efficacy remains controversial, and the mechanisms of its actions are largely unknown. Nevertheless, since acupuncture is considered to be safe and have little adverse reactions, it has been well accepted in Europe, USA, and many Asian countries. Importantly, numerous studies on patients and animal models have provided increasing evidences for its antihypertensive effects as well as its mechanisms of action. This review summarizes the common acupoints parameters used in various animal studies of hypertension including animal species and models, acupoints selection and location, stimulation parameters and effect evaluations, which will provide useful guidance for researchers when performing acupuncture in animal experiments.

ANIMAL SPECIES AND MODELS

A single animal model may not show all of the pathophysiology of diseases, thus, one disease should be investigated by using a variety of animal models. Essential hypertension in humans is a heterogeneous disease due to complex interactions between genetic and environmental factors. In order to better understand the effects of acupuncture against hypertension and its mechanisms of action, many different animal models have historically been used, including models for spontaneous hypertension, secondary hypertension, acute hypertension, and chronic hypertension. Spontaneously hypertensive rats (SHR) were developed during the 1960s by Okamoto and colleagues. Hypertension begins around 5-6 weeks of age, reaching a systolic blood pressure between 180 and 200 mm Hg by the adult stage. Hypertension models can be made by norepinephrine (NE) or epinephrine infusion via vein. Renal hypertension may be induced by the “two-kidney with one clip” protocol. In this model, one renal artery is constricted to chronically reduce renal blood flow, while the other kidney remains adequately perfused. Stress-induced hypertension is produced in conscious rats by fixing and hanging or by using the MG-2-maze stimulator. Pulmonary hypertension can be induced by hypoxia with feeding animals in a closed box for 21 days, in which oxygen concentration is maintained at 5%-11%. Hypertension is induced by renal failure by surgical removal of 7/8 of a kidney. Hypertension is also generated by the application of bradykinin to the gallbladder, which can induce a consistent sympathetic reflex, increasing blood pressure. Acupuncture has been applied exclusively to humans in the 1960s, researchers started to use the same concept of acupoints in animals, based on the theory of Traditional Chinese Medicine (TCM). The acupoints affecting blood pressure in the rat, dog, cat, and hamster are listed in Table 1.

ACUPOINTS SELECTION AND THEIR LOCATION

The selection of acupuncture points is essential for obtaining good efficacy in treatments TCM. In clinical practice and animal studies, acupoints are selected in accordance with TCM principles and diagnosis. Hypertension is a multifactorial and complicated disease, yet in clinical trials using specific acupoints has still been shown to effectively reduce blood pressure in patients with significant hypertension. Similar to human clinical studies, there are also various specific acupoint in the animal model. Table 1 is a list of major acupoints used in animal studies to reduce blood pressure, including Zusanli (ST 36), Quchi (LI 11), Neiguan (PC 6), Taichong (LR 3), Sanyinjiao (SP 6), and Hegu (LI 4). The location of these acupoints, which are based on the animal anatomy reflecting that of the human, are described in detail in Table 1. In addition, other points including Feishu (BL 13), Xinxin (BL 15), Yongquan (KI 1), Fenglong (ST 40), Shenmen (HT 7), Baihui (GV 20), and Shixuan (EX-UE 11) have been employed in animal studies as well by other studies. These studies show that each of the acupoints can be effective against hypertension. However, there are large differences in acupoints selection between human and animals, such as Taichong (LR 3), Quchi (LI 11), Fengchi (GB 20), Zusanli (ST 36), Sanyinjiao (SP 6), Baihui (GV 20), Fenglong (ST 40), Taixi (KI 3), Hegu (LI 4), Neiguan (PC 6), Ganshu (BL 18), and Xinshu (BL 15), which are frequently used in common medical practice but not in animal studies. Additionally, instead of using a single acupoint, acupuncturists often choose a combination of acupoints to treat hypertensive patients.

STIMULATION PARAMETERS

Since the effect of acupuncture, to a certain extent, depends on the adjunctive stimulation applied to acupoints on or in the skin, acupuncture treatments include a variety of techniques, such as needling, acupuncture, electricity, moxibustion, laser, and ultrasound. Each method can be effective in treating hypertension, though different stimulation parameters may lead to different physiological responses. Electroacupuncture at a low frequency of 2 Hz has been reported to significantly increase enkephalin-like immunoreactivity but not dynorphin immunoreactivity, whereas that at 100 Hz can specifically increase dynorphin immunoreactivity but not enkephalin-like immunoreactivity. Thus, stimulation parameters are important factors when considering the effect of acupuncture. Since the stimula-
The common acupoints parameters in various animal models of hypertension that can be treated by acupuncture

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Acupoint</th>
<th>Location</th>
<th>Stimulation parameters</th>
<th>Effect evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Spontaneously hypertensive rats; NE or epinephrine injection; renal hypertension; stress-induced hypertension model; hypoxia - induced pulmonary hypertension; renal failure-induced hypertension</td>
<td>Zusanli (ST 36)³⁵⁻²³, ³⁵⁻²⁰</td>
<td>Locates at between the tibia and fibula, laterally to the distal end of the cranial tuberosity of the tibia</td>
<td>Depth: 3-5 mm, Intensity: electroacupuncture: frequency 2, 4, 8 or 2/100 Hz, current 0.25-0.5, 2, 3 mA; manual acupuncture: manipulate 30 s, 2, 3 or 5 min, Time: no needle retaining, 10, 20, 15 or 30 min, Course of treatment: 7, 10, 15 or 36 d</td>
<td>Blood pressure; heart rate; tail artery systolic pressure; mean arterial pressure; systolic blood pressure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quchi (LI 11)³⁶⁻²³, ³⁶⁻²⁰</td>
<td>Lies in the depression outside the front of radial proximal joint</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taichong (LR 3)³⁶⁻²³, ³⁶⁻²⁰</td>
<td>Locates between the 1st and 2nd metatarsal of dorsal foot</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neiguan (PC 6)³⁶⁻²³, ³⁶⁻²⁰</td>
<td>Locates the gap between radius and ulna, and 3 mm away from the wrist of forelimb inside</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sanyinjiao (SP 6)³⁶⁻²³, ³⁶⁻²⁰</td>
<td>Situated approximately 3 mm proximal the largest prominence of the medial malleolus at the posterior tibia, and in the space between the Achilles tendon and the distal part of the tibia</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>NE intravenous infusion</td>
<td>Zusanli (ST 36)²⁴⁻²⁶, ²⁴⁻²³</td>
<td>Locates on 1/5 off point of dorsolateral leg, and about 1.2 cm under the fibular head, about 1 cm after tibial crest</td>
<td>Depth: 1-1.5 cm, Intensity: electroacupuncture: frequency 100 Hz, disperse-dense wave, degree of local muscle slight shake; manual acupuncture: manipulate 2 min, Low (twisting 90 degree clockwise, 60 beats per min) or mediated (1 twisting 90 degree clockwise, 20 beats per min) stimulation Time: 15 or 20 min</td>
<td>Blood pressure; Heart rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quchi (LI 11)²⁷</td>
<td>Lies in the depression at front lateral of elbow</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hegu (LI 4)²⁴</td>
<td>Locates between the 1st and 2nd metacarpal of dorsal metacarpal, and in the second metacarpal radial side of the midpoint</td>
<td>Blood pressure; Heart rate</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>NE intravenous infusion</td>
<td>Zusanli (ST 36)²⁴⁻²⁵</td>
<td>Locates on the outside of the hind leg, just below the knee, and outside of the tibial crest. The point is in the middle of the cranial tibial muscle belly</td>
<td>Intensity: 2-4 V, width of wave 0.5 ms, frequency 1-2 beat per second</td>
<td>Blood pressure; Heart rate</td>
</tr>
<tr>
<td>Hamster</td>
<td>Renal hypertension</td>
<td>Zusanli (ST 36)³¹</td>
<td>Locates on the outside of the hind leg, just below the knee, and outside of the tibial crest. The point is in the middle of the cranial tibial muscle belly</td>
<td>2 Hz, electrical current of continuous wave at 1 V intensity and 0.5 ms pulse for 30 min.</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>Cat</td>
<td>Reflex hypertension</td>
<td>Neiguan (PC 6)³⁰⁻¹⁰</td>
<td>Proximal to the carpus, on the medial face of the forearm, between the tendons of palmaris longus and flexor carpi radialis muscles</td>
<td>Output of 3 mA, and frequency 2/100 Hz for 20 min</td>
<td>Systolic and diastolic pressure</td>
</tr>
</tbody>
</table>

Note: NE: norepinephrine.
oton parameters of electroacupuncture can be precisely controlled, which makes the results easily reproducible, electroacupuncture has been employed more often in experiments compared to manual acupuncture. Table 1 shows a number of these parameters, including depth, intensity, duration, and course of treatment found to be effective in reducing blood pressure in various animal models.

The depth of inserting needle into the skin is usually determined by patient’s conditions, including size, age, and anatomical characters of the patient. For instance in a human, a needle will be often pushed only into a depth of about 1-2 mm for the hands, whereas for the buttocks, the abundance of fat and muscle allows needles to be pushed in as deep as 60-90 mm. In small animals, the depth for the buttocks is only 3-5 mm in rats and 10-15 mm in rabbits. Electroacupuncture and manual acupuncture are two main types of acupuncture used in studies on hypertension. There are currently no standard or specific stimulation parameters for animal studies. However, lower frequencies are preferred in electroacupuncture, whereas a moderate intensity of stimulation may be more effective in manual acupuncture. In addition, planning for the duration and course of acupuncture treatment may be important for obtaining better results. However, the course of treatment varies widely from 7 to 36 days depending on animal models and species.

**EFFECT EVALUATIONS**

In people 18 years or older, hypertension is defined as a systolic and/or a diastolic blood pressure consistently higher than an accepted normal value—currently <140 mm Hg for systolic, and <90 mm Hg for diastolic. The change in blood pressure following treatment directly reflects the outcome of intervention. Table 1 shows that the blood pressure value is employed to evaluate the antihypertensive effect of acupuncture in each study, including systolic blood pressure, diastolic blood pressure, and mean blood pressure. Since autonomic sympathetic tone may play a key role in the onset of hypertension, monitoring of the variability of heart rate will be essential in analyzing the causal relationship of autonomic sympathetic tone and hypertension. Some researchers suggest that the use of sphygmomanometer can provide only the values of systolic and diastolic blood pressures and does not reflect the physiological dynamic behavior of blood flow. They also propose to monitor the blood flow to better understand how blood flow is disturbed in the body, which may explain the mechanisms of efficacy of acupuncture against hypertension. Also, experimental models may enable the development of new interventions to not only decrease the blood pressure but to also reduce the organ injuries that result from hypertension. Thus, in addition to blood pressure and heart rate, we must evaluate the effects of acupuncture on all of the targeted organs and their dynamic responses to treatment.

**CONCLUSION**

Hypertension is a multifactorial disease involving complex interactions between genetic and environmental factors, and it is important to assess the effects of acupuncture on hypertension by using a variety of animal models. The rat is the most widely used species, and SHR is currently the most accepted hypertension model in studying acupuncture. The mouse may also prove increasingly useful to research of TCM, including acupuncture, with growing applications of mice as genetics models of diseases in the future. In addition, an accurate indicator of the efficacy of acupuncture treatment of hypertension is also essential.

Selection of acupoints for the treatment of hypertension is based on the diagnosis of TCM. Since hypertension is a complex condition closely associated with the heart, liver, kidney, brain, and other organs, acupuncturists often choose a group of acupoints for the treatment of patients with hypertension, which is term “acupoint prescription”. A prescription is often composed of both the main acupoints for treating the root of the disease and the accessory points for relieving the symptoms. By combining acupuncture with suitable stimulation parameters, the acupoint prescription will provide optimal effects. Nevertheless, optimal outcomes have not yet been achieved by the recent studies; we must improve the study designs, select optimal animal models, and find the best acupuncture parameters. For instance, we should employ a positive control group into these protocols.

In summary, parameters of acupuncture, such as acupuncture modalities, acupoints selection, and frequency and duration of the treatment sessions, can contribute to optimal outcome of acupuncture for the treatment of hypertension. Based on previous studies using various animal models, the optimal acupoints prescription for hypertension includes Zusanli (ST 36), Quchi (LI 11), Neiguan (PC 6), Taichong (LR 3), Sanyinjiao (SP 6), and Hegu (LI 4), where a low frequency in electrical acupuncture or a moderate stimulation in manual acupuncture is recommended. Moreover, further improvements in the indicators and tools for evaluating treatment success can provide better manipulation of acupuncture in antihypertensive therapy in both clinical cases and experimental studies.

**REFERENCES**


