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Electroacupuncture and Moxibustion Attenuate the Progression of Renal Disease in 5/6 Nephrectomized Rats

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Key Words
Kidney disease · Hypertension · Electroacupuncture · Moxibustion · Glomerulosclerosis · Proteinuria

Abstract
Background/Aim: Chronic kidney disease is a worldwide public health problem and the prevention of its progression is still a major challenge in nephrology. Specific therapies that inhibit or attenuate this process are neither available nor satisfactory. Traditional Chinese medicine has been increasingly recognized as an effective therapeutic approach in several fields of medicine. The aim of this study was to investigate the effects of electroacupuncture (EA) and moxibustion (MO) in an experimental model of progressive renal disease in rats. ***Methods:*** Twenty-one male Wistar rats were submitted to 5/6th nephrectomy (NX) and assessed 8 weeks later and were divided into three groups: NX = only 5/6 NX, NX-AS = 5/6 NX and a 20-min EA-MO session in sham points, and NX-AM = 5/6 NX and a 20-min EA-MO session in three real acupuncture points. The treatment consisted of 16 sessions twice a week. Renal function, urine volume, serum creatinine, 24-hour proteinuria, direct and indirect blood pressure, glomerulosclerosis and tubulointerstitial fibrosis indices were assessed. ***Results:*** The NX-AM group showed a significant decrease in all investigated parameters when compared to the control groups. ***Conclusion:*** Our results suggest that EA and MO attenuated the progression of renal disease in the experimental model of 5/6 NX.

Introduction

Progression of chronic kidney diseases (CKD) is a worldwide public health concern. Worldwide, five hundred million individuals are estimated to suffer some degree of CKD, and the number may double in the next 10 years [1]. The global pandemic of CKD is fueled by population ageing as well as the rise in the numbers of those affected by diabetes, obesity, and hypertension [2]. Specific and effective therapies that prevent patients from requiring dialysis or transplantation are still not available. Likewise, adequate pharmacotherapy to inhibit the progression of CKD remains a major challenge for clinical nephrologists [3].

Independent of etiology, CKD is characterized by a triad of histological hallmarks: glomerulosclerosis, inter-

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stitial fibrosis and tubular atrophy [3, 4], suggesting an ultimate common pathway [5]. Thus, relentless progression of CKD is postulated to result from a self-perpetuating, vicious cycle of fibrosis process that is activated following initial injury [6]. The possible pathophysiological mechanisms of progression of CKD include arterial hypertension, activation of the renin-angiotensin-aldosterone system, neurogenic factors, activation of various cytokines and growth factors, podocyte loss, dyslipidemia, proteinuria, and specific mechanisms of tubulointerstitial fibrosis, such as genetic factors and low nephron number [5–7]. Blocking these common mechanisms and modulation of fibrotic agents may inhibit the progression of CKD, independent of the underlying primary disease. Synergistic therapies could halt the progression of fibrosis and may potentially cause regression of any existing renal scarring [3, 8, 9].

In the last decade, a considerable number of publications have addressed the therapeutic effects of traditional Chinese medicine (TCM), and its curative concepts have been increasingly recognized as an effective therapeutic approach in several fields of medicine [10–12]. The general theory of TCM is based on the premise that there are patterns of energy flow *(Qi)* through the body that are essential for health, and disruptions of this flow are believed to be responsible for disease [13]. Medical treatments based on TCM often rely on a combination of needle insertion (acupuncture) and thermal stimulation (moxibustion) to achieve optimal results [14]. Acupuncture consists of stimulation of anatomical locations on the skin by thin, solid, metallic needles, which are manipulated manually or with the help of an electrical device. Stimulation of these areas by heat is called moxibustion (MO), a technique that applies heat to acupoints by burning a compressed, powdered, combustible mass obtained from the ground young leaves of an Eurasian artemisia *(Artemisia vulgaris)* [13–15].

Investigators have demonstrated that the nervous system, neurotransmitters and endogenous substances are stimulated or inhibited by needle stimulation, electroacupuncture (EA) and MO [16–20]. EA is an electrical stimulation through the acupuncture needles with short current pulse at different frequencies [11]. Both EA [10, 21–24] and MO [25] have been shown to influence renal function, although little is currently understood about their role [26].

Acupuncture has been used in the treatment and prevention of cardiovascular diseases and hypertensive syndromes [27–30]. Hypertension is an important presenting feature of renal disease and is probably one of the most important factors contributing to the progression of CKD [6, 31, 32]. Recent studies have demonstrated that EA on the stomach at the 36th point (ST-36) induces upregulation of neuronal nitric oxide synthase (NOS) expression in the gracile nucleus and medial nucleus tractus solitarius, and this can modify central cardiovascular regulation [33, 34]. Kim et al. [35] demonstrated that EA on ST-36 reduces blood pressure by modulation of endothelial NOS and neuronal NOS expression.

Therefore, the aim of the present study was to investigate the influence of EA and MO on a progressive experimental CKD model.

Methods

Animals

All experimental procedures were conducted according to the National Institutes of Health guidelines for use and care of animals, and the study protocol was approved by the Ethics in Research Committee of the Universidade Federal de Sao Paulo (process No. 0668/06). Twenty-one male Wistar rats (250–300 g) were obtained from the animal care facility of our institution. The animals were divided into three groups and the entire treatment consisted of 16 sessions distributed along 8 weeks (20-min session, twice a week). The treatment plan was based upon published works in the literature [14–16]. The animals were housed in group cages, given access to rat chow and water ad libitum and maintained in a temperature-controlled environment (23°C) on a 12-hour light/dark cycle.

Experimental Protocols

After a 7-day adaptation period, the animals were weighed and housed in metabolic cages kept in a humidity- and temperature-controlled room for 24 h to collect urine. The following day, rats were randomly submitted to five sixth nephrectomy (5/6 NX) under anesthesia with ketamine (100 mg/kg i.p.) plus xylazine (10 mg/kg i.p.). Briefly, after ventral laparotomy, removal of the right kidney and ligation of 2 branches of the left renal artery were performed, resulting in infarction of two thirds of the left kidney.

The animals were divided into three groups and assessed 8 weeks later: control (NX) = only 5/6 NX; sham EA-MO (NX-AS) = 5/6 NX and a 20-min EA-MO session in sham points 2 times a week, and EA-MO (NX-AM) = 5/6 NX and a 20-min EA-MO session on real acupoints 2 times a week.

Proteinuria and Renal Function

Urinary protein excretion was measured using the Sensiprot Protein Assay Kit (Labteste Diagnostica, Brazil), while serum creatinine was measured using the Labteste Creatinine Kit (Labteste Diagnostica). An initial, baseline measurement of body weight and urine volume was made.

Table 1. Body weight, diuresis, proteinuria and serum creatinine prior to treatment and at the conclusion of the study

Group (n = 7)	Body weight, g		Diuresis, ml/24 h		Proteinuria, mg/24 h		Serum creatinine, mg/dl	
	initial	end	initial	end	initial	end	initial	end
NX	270 ± 11	320 ± 10	10.0 ± 1.4	30.7 ± 3.8*	12 ± 3.2	139.1 ± 25.2*	0.4 ± 0.1	1.5 ± 0.3*
NX-AS	269 ± 6	324 ± 12	10.5 ± 1.3	32.7 ± 2.4*	14.3 ± 4.4	161.5 ± 56.8*	0.4 ± 0.1	1.4 ± 0.3*
NX-AM	272 ± 7	326 ± 10	10.0 ± 0.5	23.2 ± 3*, +	13.8 ± 3.7	78.4 ± 5.0*, +	0.4 ± 0.1	0.7 ± 0.1*, +

Values are means ± SD. * $p < 0.05$ vs. initial parameters; + $p < 0.05$ vs. final NX and NX-AS.

Systolic Blood Pressure

The systolic blood pressure (SBP) was monitored in conscious rats by the tail cuff method [36] (PE300; Narco Bio-Systems) once a week.

Mean Arterial Pressure

Animals were anesthetized with urethane (1.2–1.4 g/kg i.p.) and the left femoral artery and vein were cannulated. Supplemental doses of α-chloralose (10 mg/kg i.p.) were given as necessary to maintain an adequate depth of anesthesia, as assessed by animals' lack of response to noxious toe pinch, a respiratory pattern that followed the ventilator, and stable blood pressure. Body temperature was monitored with a rectal probe (Letica Scientific Instruments) and was maintained between 36 and 37°C by a thermostatically controlled heating pad. Mean arterial pressure (MAP) was measured with a cannnula inserted into the femoral artery that was connected to an on-line system McLab (ADI Instruments).

EA and MO

The localization of the acupoints was based on rat anatomical references [37, 38]. A number of papers studying different animal species have clearly shown that the location of acupuncture points follows a similar distribution in different mammals [39]. The needle used for stimulation was 0.25 mm in diameter and 2 cm long, made of a stainless steel stem and copper handle. For bilateral points, the positive output lead from the EA apparatus was connected to the left acupoint and the negative terminal was connected to the right acupoint and the polarity between these points was continuously alternated.

The NX-AM group received acupuncture at points located on the hind limbs. In the rat, ST-36 *(Zusanli)* is located approximately 1 mm lateral to the tibial tuberosity, whereas KI-3 *(Taixi)* is located in the medial border of the tibia, 0.5 mm above the medial maleolus, both of which are easily located by manual inspection. The needles were bilaterally inserted at a depth of approximately 0.5 mm at the ST-36, and just above the skin at KI-3 (due to the lack of evident muscular mass under this area) and then subjected to EA (20 Hz/1 V, Plexus AP585; VWV Biotherapy/Lautz, Brazil) for a period of 20 min. MO stimulation was applied at the BL-23 *(Shenshu)* acupoint, for a period of 2 min concomitant with EA.

The choice of these acupoints was based on their reported abilities to produce hypotensive responses as well as to participate in the central regulation of renal and cardiovascular responses [16, 18, 22, 25, 29]. *Zusanli* (ST-36) point also promotes analgesic and anti-inflammatory effects. On the other hand, *Shenshu* (BL-23) and *Taixi* (KI-3) points are important in any kind of kidney deficiency due to their tonifying effect on kidney *Qi* [20, 30].

The NX-AS group received sham acupuncture at points located on neighboring skin areas (less than a 1.5-cm distance from the real acupoints) for which impedance analysis did not reveal a lower skin resistance compared to adjacent areas. The sham points were localized in regions with different nerve distribution as ST-36, KI-3 and BL-23. The needles were bilaterally inserted at a depth of approximately 0.5 mm and then subjected to EA (20 Hz/1 V, Plexus AP585; VWV Biotherapy/Lautz) for a period of 20 min. MO stimulation was applied on neighboring skin area of the BL-23 acupoint, for a period of 2 min concomitant with EA. In sum, the stimulation time was the same for both groups.

Renal Histological Studies

Portions of the remnant kidneys were fixed in 10% neutral formalin. Paraffin sections (3 μm in thickness) were cut and stained with HE, periodic acid-Schiff reaction and Masson's trichrome.

All morphological evaluations were performed in a blinded manner by a single observer. Glomerular damage was evaluated on the basis of the percentage of glomeruli that were sclerotic or collapsed. Glomerulosclerosis was defined as segmental increases in the glomerular matrix, segmental collapse, obliteration of the capillary lumina, and accumulation of hyaline, often with synechial attachment to Bowman's capsule [40]. The extension of glomerulosclerosis and glomerular collapse was evaluated in each kidney by consecutive examination under light microscopy. Tubulointerstitial injury was defined as inflammatory cell infiltration, tubular dilation and/or atrophy, or interstitial fibrosis [3, 31]. Injuries were examined at least in 20 areas by the following scoring system [41]: 0 = changes <10% of the cortex, 1+ = changes in up to 25% of the cortex; 2+ = changes in up 50% of the cortex; 3+ = changes in >50% of the cortex sections.

Statistical Analysis

Results are presented as mean ± standard deviation. Comparisons among different groups were evaluated using multiple analyses of variance (ANOVA) followed by a post-hoc protected least-significant difference test. The level of statistical significance was defined as $p < 0.05$.

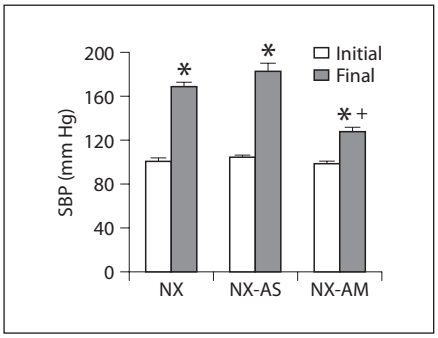

Fig. 1. SBP. * $p < 0.05$ vs. initial; $^{+}$ $p < 0.05$ vs. final NX and NX-AS.

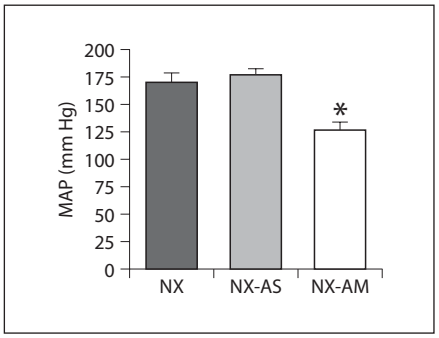

Fig. 2. MAP. * $p < 0.05$ vs. NX and NX-AS.

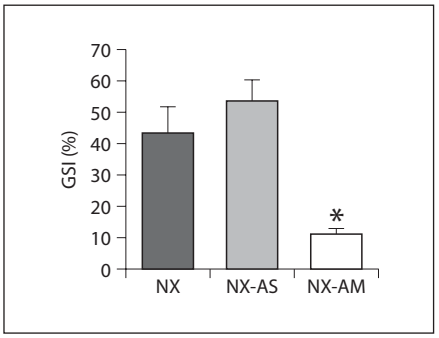

Fig. 3. GSI. * $p < 0.05$ vs. NX and NX-AS.

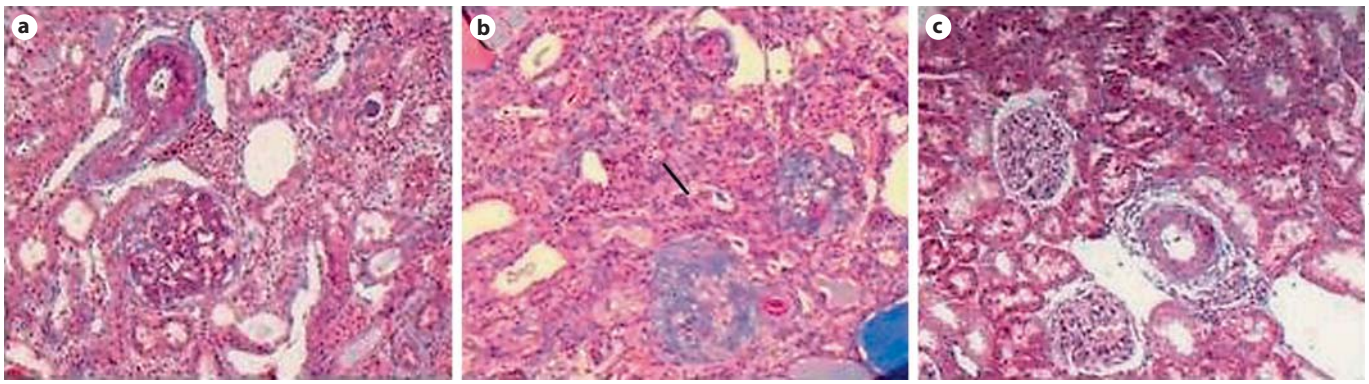

Fig. 4. Light micrographs of the kidney from rats of the several experimental groups: NX (**a**), NX-AS (**b**) and NX-AM (**c**). Widespread glomerulosclerosis and tubulointerstitial injury can be seen in both **a** and **b**, but EA-MO-treated rats (**c**) have significantly less renal injury. Masson's trichrome. ×100.

Results

EA and MO Significantly Reduced Diuresis, Proteinuria and Serum Creatinine

As shown in table 1, the experimental groups did not differ in relation to the body weight at 8 weeks. The final urine volume was significantly higher compared to the beginning in all the nephrectomized groups. However, when we compared the final urine volume among the different groups, we found that it was significantly reduced in the NX-AM group ($p < 0.05$).

At the end of the experiments, proteinuria and serum creatinine concentration were significantly higher in relation to initial parameters. However, the NX-AM group had a significantly lower proteinuria and serum creatinine concentration in relation to the other groups ($p < 0.05$).

EA and MO Significantly Reduces SBP and MAP

The initial SBP was similar in all four groups of nephrectomized rats. At the end of the study, EA and MO treatment significantly attenuated the elevation of SBP and MAP (mm Hg) in relation to the untreated groups ($p < 0.05$) (fig. 1, 2).

EA and MO Reduces Glomerulosclerosis and Tubulointerstitial Fibrosis Indices

As shown in figure 3, the glomerulosclerosis index (GSI) was higher in all NX groups, although the GSI was lower in the NX-AM group compared to the other groups ($p < 0.05$). As shown in figure 5, the tubulointerstitial fibrosis index grade in the NX-AM group was much lower compared with the other groups ($p < 0.05$). Figure 4 presents typical histopathological specimens for each experimental group.

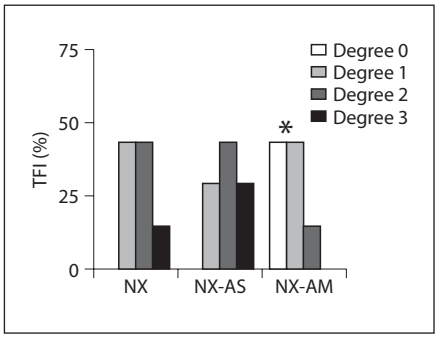

Fig. 5. Tubulointerstitial fibrosis index (TFI). * $p < 0.05$ vs. NX and NX-AS.

Discussion

In the present study, we investigated the renal effects of EA and MO in a rat model of the progression of CKD. The progression of CKD is one of the most-researched topics in nephrology. Although there have been many advances in the elucidation of renal physiology and pathophysiology, current efforts still cannot completely abrogate its progressive nature, even when appropriate therapies are imposed.

Experimental evidence shows that several pathophysiological mechanisms are involved in progression of kidney disease, including a complex interaction between hemodynamic and inflammatory factors [31], occurring simultaneously in renal tissue. These factors include glomerular hypertension and hyperfiltration triggering expression of chemokine in renal cells and resulting in infiltration of macrophages, fibroblasts and myofibroblasts. This infiltration leads to an amplified inflammatory process with further production of cytokines and chemokines, and consequently spreading of the process [6]. In addition, systemic arterial hypertension may contribute to the aggravation of the injury.

There have been some clinical and experimental studies reporting beneficial effects of acupuncture and MO on arterial hypertension [25, 28, 30, 35] and the inflammatory process [15]. Therefore, we undertook this work to address the effects of the combination of EA stimulation applied to the *Zusanli* (ST-36) and *Taixi* (KI-3) acupoints, and MO at the *Shenshu* dorsal acupoint (BL-23) on the progression of renal disease in a model of progressive disease in rats. The new findings of this study are: (1) improved renal function demonstrated by a significant reduction of diuresis, proteinuria and serum creatinine, (2) a significant reduction of systemic arterial hypertension, and (3) attenuation of the fibrosis process. This is the first evidence demonstrating that EA stimulation and MO improve renal function and attenuate the progression of the renal disease in the experimental model of 5/6 NX in rats. Ma [24] and Holub [42] reported that EA positively influenced the maintenance of the capacity of urinary concentration. Lee et al. [25] investigated the effects of MO at BL-15 and BL-27 (bladder) acupoints and demonstrated that those acupoints could modulate water homeostasis by interfering with secretion of hormones such as renin, aldosterone and atrial natriuretic peptide. In addition, these authors found that SBP decreased after MO at the meridian point BL-15.

Holub [42] demonstrated that treatment of patients with glomerulonephritis with acupuncture presented positive effects on renal function resulting in decreased proteinuria and erythrocyturia, as the blood biochemical spectrum was returned to a normal level. Also, arterial pressure was normalized in patients with mild hypertension or reduced in those with moderately severe to severe hypertension. Chen et al. [43] showed that acupoint thread implantation associated with Chinese herbs improved renal function and led to a decrease in PTH expression and inhibition of TGF-β_1 expression. In addition, Garcia et al. [26] reviewed the potential effects of acupressure techniques in renal inflammation and whether these effects could be mediated through the newly identified cholinergic anti-inflammatory pathway. The anti-inflammatory actions of acupuncture possibly occur via the reflexive central inhibition of the innate immune system and are mediated by the efferent vagus nerve restraining macrophages from their inflammatory action, resulting in diminished production of TNF, IL-1β, IL-6, IL-18, and augmented anti-inflammatory (IL-10) cytokines, thus controlling the extent of inflammatory response [44, 45].

One of the most noticeable findings of our study was the beneficial effect on systemic blood pressure. All animals developed systemic hypertension, while animals treated with EA and MO exhibited significant lower blood pressures. Among the likely mechanisms that would justify this result is the inhibition of the sympathetic system by acupuncture, which has already been shown in a large number of studies [46–48]. The effects of EA and MO on arterial hypertension are described in other experimental models and clinical trials. Wu et al. [49] showed that acupuncture at VB-34 *(Yanglingquan)* results in the reduction of the SBP and prevented the hypertrophy of the heart muscle cells in spontaneously hypertensive rats (SHR). Similarly, EA at ST-36 *(Zusanli)* for

20 min promoted a depressant effect on the MAP in rats with induced hypertension, mediated by NO in periaqueductal gray substance through activation of inhibitors of the sympathetic system and the mitigation of cardiac activity [18, 29, 33]. MO at BL-15 *(Xinxhu)* and BL-27 *(Xiaochangshu)* points in SHR rats resulted in a decrease in MAP [25]. In another trial, acupuncture was an effective and safe therapeutic modality for treatment of mild to moderate hypertension in adult patients [30].

Finally, our results suggest that the lowering effect on proteinuria and blood pressure, two parameters objectively measured and of great relevance to the progression of CKD, exerts a beneficial influence on this process. Ultimately, this is expressed by preventing an increase in serum creatinine, as well as by reducing urinary volume and the degree of glomerular and tubular damage.

Despite being an ancient science, acupuncture and MO remain contemporary. Social and historical validations of acupuncture and TCM have been confirmed in recent decades, when a considerable amount of publications scientifically proved the traditional method and added new clinical applications. In the past 30 years, scientific advances, particularly in the field of neuroscience, have clarified the neurobiological bases for TCM. The clinical validation of acupuncture is consolidated through modern research methodologies tracing the development of the quality criteria in clinical trials and in the laboratory.

This work is pioneering in the field of nephrology, although further research must be performed to increase the understanding of the mechanisms responsible for this positive action of acupuncture and MO in the progression of CKD. Since CKD is a result of multifactorial aspects, some of them unknown, it is not simple to accomplish a unified comprehensive treatment. Therefore, current therapeutic strategies are directed toward specified goals contributing to this syndrome, mainly high blood pressure and proteinuria and associated comorbidities, such as cardiovascular disorders and activation of inflammatory cascade [2, 3, 6]. There is already evidence in literature on the beneficial cardiovascular effects of acupuncture as well as its anti-inflammatory effect. So, acupuncture might be an ancillary therapeutic strategy and could be integrated with conventional treatments for patients with CKD [10, 24, 26, 42].

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