

침 자극의 씨클로포스파미드로 유발한 백서 방광염 모델에서 방광의 과활동성에 대한 억제효과

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Acupuncture attenuates bladder hyperactivity in the cyclophosphamide-induced cystitis in rats.

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Abstract

목적 : 씨클로포스파미드(Cyclophosphamide, CP)는 많은 중앙 질환에서 단독 혹은 다른 화학요법과 함께 사용되는 항암제이다. CP의 독성과 부작용으로 출혈성 방광염이 많은 문제를 일으키고 있다. CP로 유도된 방광염 모델은 하부요로의 통증질환을 이해하기 위한 내장성 통증의 주요한 모델로 사용되고 있다. 본 연구는 암컷 백서에서 CP로 유도된 방광의 과활동성에 대한 침의 효과를 관찰하기 위해 요류역학검사를 수행하였다.

방법 : CP (200 mg/kg) 투여 18시간 후에, 지속적 생리식염수 관류 (분당 0.04 ml)로 방광내압 측정을 통해 방광기능을 평가하였다. 관류 2시간 이후부터, 위중/근륜 100 Hz, 위중/근륜 2 Hz, 족삼리/삼음교 100 Hz, 족삼리/삼음교 2 Hz 전침 자극을 하여 자침 전후의 방광수축간격(intercontraction interval, ICI)을 비교하였다.

결과 : 방광내압 측정하는 동안, 방광수축간격은 CP로 유도된 방광염 모델에서 정상군에서 보다 유의하게 짧았다. CP로 유도된 방광염 모델에서, 위중/근륜100Hz 전침 자극은 자침 전보다 통계적으로 유의하게 40.6 ± 10.9 % 증가시켰고, 2Hz 전침 자극은 자침 전보다 현저하게 22.1 ± 13.7% 증가시켰다. 반면에, 족삼리/삼음교 전침 자극은 100Hz, 2Hz 전침 자극 각각 13.7 ± 8.1 %, 8.7 ± 4.5% 약간 증가시켰다.

결론 : 족태양방광경의 경혈에 100Hz 전침 자극을 하여 CP로 유도된 방광의 과활동성을 억제하였다. 이러한 결과는 침 자극으로 CP로 유도된 혹은 다른 종류의 방광염을 치료하는데 효과적인 것임을 시사한다. 또한, 특정 경락의 경혈과 해당 장부의 질환의 치료에 연관성을 보여준다.

Key words : cystitis, cystometry, bladder meridian, cyclophosphamide

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I. Introduction

Cyclophosphamide (CP), as an alkylating antineoplastic chemotherapeutic agent in the nitrogen mustard group, is a drug with a wide spectrum of clinical uses, and it has been proved to be effective in the treatment of many neoplastic diseases¹⁾. However, there are major adverse effects of CP therapy, including hemorrhagic cystitis, which is difficult to treat²⁾. Recent experiments involving a chemically CP-induced urinary bladder inflammation have demonstrated alterations in neurochemical³⁾, organizational⁴⁾, and electrophysiological⁵⁾ properties of micturition reflex pathways. CP-induced cystitis has been regarded as a model of cystitis and visceral pain to gain an understanding of painful lower urinary tract syndromes^{6,7)}.

Several clinical studies have been performed in the West concerning acupuncture, as a treatment of bladder dysfunction⁸⁾. It was demonstrated that acupuncture attenuated urinary incontinence and urgency caused by bladder overactivity or instability, and improved urodynamic measurements such as bladder capacity^{9,10)}. It was found that an inhibitory effect on rhythmic micturition contractions (RMCs) was elicited by acupuncture-like stimulation¹¹⁾. It was also re-

ported that electroacupuncture at Hoku (LI4) with different stimulation frequency modulated the hyperactive bladder by eliciting a distinct mechanism to activate the sympathetic nervous system¹²⁾. Moreover, an EA-drug combination was more effective in reducing cyclophosphamide-induced emesis in the ferret¹³⁾. However, the mechanism of acupuncture's success in treating CP induced cystitis rats remains unclear.

The present study was therefore designed to investigate the effect of the two different frequencies of the electroacupuncture at the specific acupuncture points on the changes in cystitis models induced by CP using the urodynamics study.

II. Materials and Methods

1. Animals

Twenty-four Spague-Dawley female rats were purchased from Samtaco (Kyunggi, Korea). The rats weighed 200g-220g and were housed in a temperature-controlled room with a 12 h light/dark, and access to food and water ad libitum. The rats were acclimated for 1 week before use. All experiments were carried out in accordance with a protocol approved by the Kyung Hee University

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Table 1. The comparison of EA stimulation at two kinds of combination of acupoints on the intercontraction interval using cystometry.

Group	The intercontraction interval (sec)	
	Pre-EA	Post-EA
100 Hz EA at BL40/BL60	131.7 ± 13.3	178.2 ± 20.7 *
2 Hz EA at BL40/BL60	139.7 ± 16.8	170.0 ± 23.9
100 Hz EA at ST36/SP6	140.8 ± 24.5	163.2 ± 28.9
2 Hz EA at ST36/SP6	149.7 ± 21.1	165.3 ± 27.0

The values are expressed as means ± S.E. Differences were considered statistically significant at P < 0.05. EA: electroacupuncture.

Animal Care and Use Committee.

2. Surgical procedure

Rats were injected with cyclophosphamide (200mg/kg, i.p.) 18 h prior to cystometrogram study. The urinary bladder was catheterized using the method of Yaksh et al¹⁴. The bladder was exposed via a mid-line abdominal incision. A catheter (PE-50), the bladder end of which was heated to create a collar, was inserted through a small incision in the bladder dome, and a suture was tightened around the collar. The other end of the catheter was passed through subcutaneous tissue and exited through the skin. After closing the abdominal incision by suturing the muscle and skin, rats that were to be studied without anesthesia were placed in a restraining cage for 5-6 h (including 2 h of recovery from halothane) that was large enough to permit them to adopt a normal

crouching posture but narrow enough to prevent them from turning around. The rats were subsequently allowed to recover from halothane anesthesia.

3. Cystometrogram

The bladder catheter was connected via a T-stopcock to a pump for continuous infusion of physiological saline and to a pressure transducer. Physiological saline was infused at room temperature into the bladder at a constant rate of 0.04 ml/min to elicit repeated voiding responses and the intravesical pressure was recorded continuously. The volume-evoked micturition reflexes were studied in conscious, restrained rats. After the cystometrogram had become stable (approximately 2 hr after starting the infusion of saline), the urodynamic parameters were measured for 30 min before and after EA stimulation (Fig. 1).

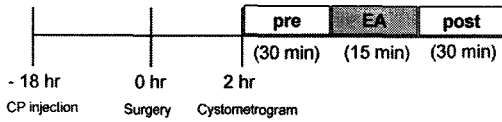


Fig. 1. The experimental protocol was as shown. Rats were injected with cyclophosphamide (200mg/kg, i.p.) 18 h prior to cystometrogram study. After the cystometrogram had become stable (approximately 2 hr after starting the infusion of saline), the urodynamic parameters were measured for 30 min before and after electroacupuncture stimulation. EA: electroacupuncture, Pre: 30 min before EA, Post: 30 min after EA.

4. Acupuncture stimulation

The anatomical locations of stimulated acupuncture points in rats were equivalent to the acupoints in man as described by Stux and Pomeranz¹⁵⁾. Two interdermal needle soldered to a flexible electrical wire, were inserted vertically into the selected acupoints. Acupoints BL40 and BL60 were selected for main acupoints, as one of the five-phase acupoints in the *Bladder meridian*. ST36 and SP6 were selected for the control acupoints in the other meridian. The stimulus consisted of 2 Hz electroacupuncture (i.e. low frequency electroacupuncture, LF EA) or 100 Hz electroacupuncture (i.e. high frequency electroacupuncture, HF EA) constant current electro-stimulations (HANS LH202H, Neuro-

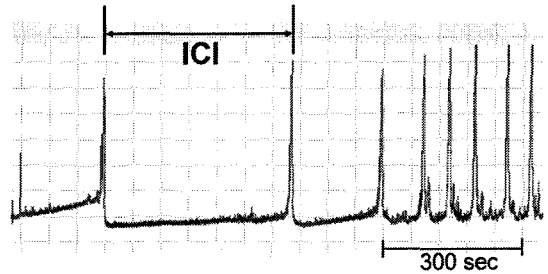


Fig. 2. Cystometrogram recordings in rats injected with CP. Intercontraction interval (ICI) were measured using cystometrogram. ICI: intercontraction interval.

science Research Center, Peking University, Beijing, China) for 15 min approximately 2 hr after starting the infusion of saline (Fig. 2).

5. Statistical analysis

The values are expressed as means \pm S.E. Data were compared between before and

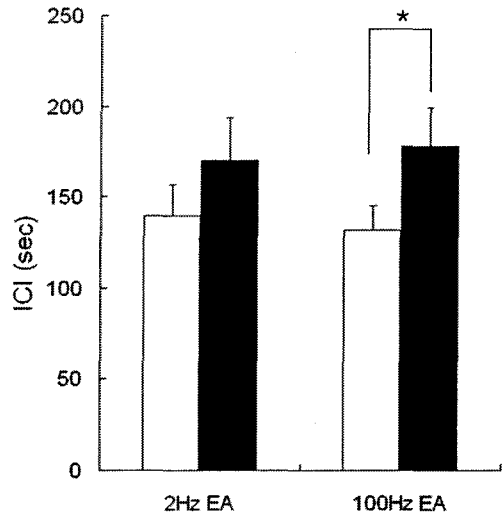


Fig. 3. HF EA at acupoints BL40/BL60 significantly increased the ICI, but LF EA slightly increased the ICI. The values are expressed as means \pm S.E. Differences were considered statistically significant at $P < 0.05$.

after electro-acupuncture stimulation using paired *t*-test. Differences were considered statistically significant at $P < 0.05$.

III. Results

The comparison of EA stimulation at two kinds of combination of acupoints on the intercontraction interval using cystometry was performed (Table 1). During continuous infusion cystometry, the intercontraction interval (ICI) was increased to 178.2 ± 20.7 sec from 131.7 ± 13.3 sec by 40.6 ± 10.9 % in the 100 Hz EA at acupoints BL40/BL60 group, and markedly increased to 170.0 ± 23.9 sec from 139.7 ± 16.8 sec by 22.1 ± 13.7 %(Fig. 3). However, 100 Hz EA at acupoints ST36/SP6 did not significantly enhance the ICI to 163.2 ± 28.9 sec from 140.8 ± 24.5 sec by 13.7 ± 8.1 %, and 2 Hz EA at acupoints ST36/SP6 slightly enhanced the ICI to 165.3 ± 27.0 sec from 149.7 ± 21.1 sec by 8.7 ± 4.5 (Fig. 4).

IV. Discussion

Our preliminary study showed the consistency with the previous other studies, indicating intercontraction interval (ICI) was smaller in CP-injected rats than in the control rats during continuous infusion cystometry¹⁶⁾.

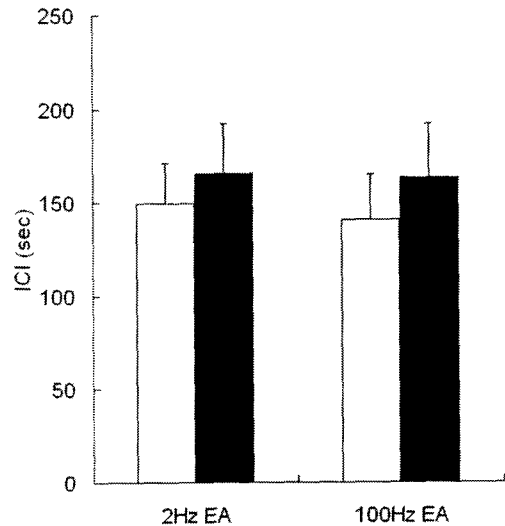


Fig. 4. Both HF and LF EA at acupoints ST36/SP6 did not significantly enhance the ICI. The values are expressed as means \pm S.E.

The present study demonstrated that HF EA at the acupoints on *Bladder meridian* resulted in a significant increase of the intercontraction interval (ICI) in the cyclophosphamide-induced cystitis, while LF EA at the acupoints on *Bladder meridian* produced slight increase of the ICI of the rats. On the other hand, EA at the acupoints on the other meridian did not show significant increase of the ICI in the CP-induced cystitis.

In the present study, acupuncture at *Bladder meridian* resulted in increased intercontraction interval (ICI) in cyclophosphamide-induced cystitis rats. These restorations are in accordance with several human studies⁸⁾. However, unlike acupoints BL40/BL60 on the *Bladder meridian*, ST36/SP6 acu-

puncture treated group showed no significant changes. These results can be explained by the fact that each acupoint exhibits different effects. The acupoints BL40/BL60 have been used to treat urinary disorders¹⁷⁾, while acupuncture point ST36/SP6 has been used to relieve pain and modulate nicotine addiction^{18,19)}. It was demonstrated that stimulation of acupoints ST36/SP6 or GB34/BL57 in the same spinal segments induced distinct though overlapped cerebral response patterns, indicating the existence of acupoint specificity using functional magnetic resonance imaging²⁰⁾. Similarly, in the current study, with stimulation of acupoints innervated by the same spinal segments, we demonstrated that EA at these specific pairs of acupoints on the *Bladder meridian* inhibited the bladder hyperactivity in the CP-induced cystitis animal model. At least but not at last, our results provide an urodynamic evidence for the existence of acupoint specificity, though further proofs are still necessary to elucidate this phenomenon.

Our data demonstrated that HF EA at *Bladder meridian* most prominently inhibited bladder hyperactivity in CP-induced cystitis rats model, not in LF EA at the same meridian. Enkephalins injected intracerebroventricularly have been demonstrated to re-

duce the volume when the pontine micturition reflex occurs²¹⁾. Endorphins seem to have an effect to increase the storage capacity of the bladder. Chen *et al.* suggested that sharing the same central mechanism with LF EA to activate mu and delta opioid receptors, HF EA was able to activate kappa receptors which were not excited by LF EA, which means that EA at various frequencies might exerts different response at the same stimulation site²²⁾. It was also found that functional activities of certain brain areas were correlated with the effect of EAS-induced analgesia, in a frequency-dependent dynamic²³⁾. Similarly, different patterns of inhibitory effect were elicited by LF EA and HF EA in the present study might be derived from the different central mechanisms. However, the precise neural pathway and the centers involved in this reflex need more experiments to be clarified.

In the present study, we examined urodynamically the effect of acupuncture on the bladder function in cystitis rats induced by CP using cystometrogram. To sum up, electroacupuncture at the acupoints on Urinary Bladder meridian produced the increase of the intercontraction interval of the CP-induced cystitis. These results suggest that the appropriate acupuncture treatment may be

valuable for alternative therapies for the treatment of cystitis. However, clinical studies are needed to confirm these findings.

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