



Comparison the Analgesic Effect of Electeroacupuncture and Tramadol on Writhing Test in Rat

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ABSTRACT

Background: Since the use of electeroacupuncture (EA) may be suitable for animals in veterinary medicine, in present study, analgesia of this technique on control of visceral pain was compared to tramadol effect. **Methods:** The study was carried in 5 groups of Wistar rats. One group was kept without restriction and drugs (group1) and another group was only restricted (group 2). Acetic acid was intraperitoneally injected in 3 to 5 groups of rats (10 rats each group) and the number of writhes was counted for 40 minutes after acetic acid injection. Group 3 received acetic acid and was kept as control. Tramadol was administered at dose 10 mg/kg intramuscularly in group 4 and rats of group 5 received EA and ST-36 (Zu San Li) and SP-6 (San Yin Jiao) points were stimulated at 1ms, 0.3 and 0.3 Hz, duration, intensity and frequency respectively. The amount of serum cortisol was measured by ELISA kit at end of study. **Results:** The results shown the mean of writhing was significantly decreased in group 4 and 5 with comparison to group 3. In groups 3, 4 and 5 the mean writhing was 22.3, 3.4 and 3.6 per 10 minutes respectively. The mean of serum cortisol was greater in group 2 than other groups. This mean was significantly was lesser in group 4 and 5 in comparison to control group 3. In other hands, the mean cortisol was 0.7 and 1.16 ng/ml in groups 3, 4 and 5 respectively. **Conclusion:** Present study shows that tramadol and EA has similar analgesic effect on writhing test in rats.

Introduction

Acupuncture analgesia is a useful clinical technique for pain relief, and it has been using in treating diseases and pain and “Zusanli” point (St. 36) is one of the 365 classical acupuncture points.¹ Electeroacupuncture (EA) produces analgesia by endogenous substances including opioids.² Also EA is one of the most desirable methods for the management of pain including chronic visceral pain and it is easy to apply, the cost is low and its adverse effects are minimum.³ Acupuncture can be an effective treatment in acute herpes zoster pain.⁴ EA was used in laboratory animals and the rat has 13 thoracic, six lumbar, four sacral, and 28 coccygeal vertebrae acupoints.⁵ Tramadol, a centrally acting analgesic structurally related to codeine and morphine, inhibits serotonin and norepinephrine reuptake, enhancing inhibitory effects on pain transmission in the spinal cord.⁶ Acetic acid is an animal model for acute and tonic peripheral visceral pain in rats and mice. It induces a stereotypic response pattern in form of constrictions composed of abdominal contractions, twisting and turning of the trunk and extension of the hind limbs, known as the writhing test.⁷

Since the use of EA may be suitable for animals in veterinary medicine, effect of this technique on control of visceral pain was compared to tramadol in present study.

Materials and Methods

Fifty healthy Wistar female rats, 10-12 weeks of age, weighing 180-200g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed polycarbonate cage for a 2-week acclimation period. Rats were fed *ad libitum* by standard laboratory pellet (Pars khurakdam, Shushtar, Iran.) and tap water. A 12-h light: 12-h dark cycle was maintained. Room temperature was at 23±2 °C. The study was carried in 5 groups of rats. One group was kept without restriction and drugs (group1) and another group was only restricted (group 2). Acetic acid (1% solution) was intraperitoneally injected 1ml/rat⁸ in group 3 to 5 of rats (10 rats each group) and the number of writhes was counted for 40 minutes after acetic acid injection. Group 3 received acetic acid and was kept as control. We used EA apparatus from XWD-808 Multipurpose Health, Device instruction manual and EA-niddles

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from Suzhou Acupuncture & moxibustion co. ltd-China. Twenty minutes after acetic acid injection, tramadol (from Exir Co. Iran) was administered at dose 10mg/kg intramuscularly in group 4 and rats of group 5 received EA and ST-36 (Zu San Li) and SP-6 (San Yin Jiao) points were stimulated at 1ms, 0.3 and 0.3Hz, duration, intensity and frequency respectively.² The amount of serum cortisol was measured by ELISA kit (Radim Co. Iran) at end of study.

Statistical significance between groups was determined using SPSS program (version 16, USA). Significance of differences between groups were assessed using one-way analysis of variance (ANOVA) followed by least

significant difference (LSD) post hoc comparison. The minimum level of significance was $p < 0.05$.

Results

The results shown the mean of writhing number was significantly decreased in group 4 and 5 with comparison to group 3. In groups 3, 4 and 5 the mean (\pm SE) writhing was 22.3 ± 3.67 , 3.4 ± 1.13 and 3.6 ± 0.8 per 10 minutes respectively in 2th, 3th and 4th count times (Table1). The EA and tramadol had similar analgesic and anti-inflammatory effect in writhing test in rat.

Table 1. Mean \pm SE. number of writhes/ 10minutes in rats. (Different letters show significant difference between groups).

Groups	Number of writhes after acetic acid injection			
	0-10 (min)	10-20 (min)	20-30 (min)	30-40 (min)
Only acetic acid	9 ± 1.39^a	22.3 ± 3.68^a	17.5 ± 2.24^a	14.9 ± 1.7^a
Acetic acid \pm Tramadol	2.9 ± 0.77^b	3.4 ± 1.13^b	4.3 ± 1.51^b	6.1 ± 1.12^b
Acetic acid \pm electroacupuncture	7.1 ± 1.04^a	3.6 ± 0.8^b	6.7 ± 1.18^b	6 ± 1.13^b

The mean of serum cortisol was greater in group 1 (1.36 ± 0.04 ng/ml) than other groups. This mean was significantly was lesser in group 4 and 5 in comparison to control group 3. In other hands, the mean (\pm SE) cortisol was 0.7 ± 0.04 and 1.16 ± 0.07 ng/ml in groups 3, 4 and 5 respectively (Figure 1).

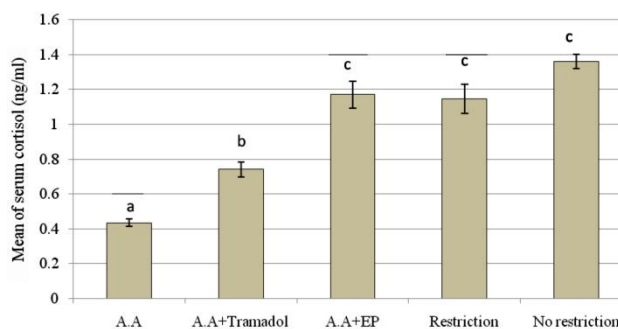


Figure 1. Mean \pm SE. of serum cortisol of rats: A.A: only acetic acid; A.A+Tramadol: acetic acid+tramadol; A.A+EP: acetic acid+electroacupuncture; Restriction: only restriction; No restriction: without restriction. The letters show significant difference between groups of rats ($p < 0.05$). $n = 10$.

Discussion

At present study, we used acetic acid model for anti-inflammatory effect of EA and tramadol. The acetic acid induces inflammation in peritoan and causes writhing position.

The endogenous opioid system and nonopioid systems may be involved in EA-induced analgesia because administration of opioid, serotonin and catecholamine antagonists including alpha-2 antagonists block EA-induced analgesia.^{2,9} We used electroacupuncture at "Zusanli" (ST.36) and "Sanyinjiao" (SP.6) points. These points are frequently used in animal research to study acupuncture effects on various physiological

mechanisms and control systems changes including gastrointestinal disorders and visceral pain.¹⁰

There is evidence showing that EA stimulates the pain inhibitory system at the spinal cord, brain stem and other areas of the central nervous system, such as thalamus, 3rd ventricle, mesencephalon, diencephalon, hypothalamus, and hypophysis. Endogenous opioids, such as endorphins, enkephalins, and dynorphins have also been well documented to contribute to the analgesic effect of acupuncture. The dense-disperse mode of electrical stimulation, with the frequency ranging from 10 to 1000 Hz, used in this study probably produced the release of a combination of endogenous opioids. Fang and et al (2009) demonstrated electroacupuncture at ST.36 and SP.6 decreased production of cytokines in experimental arthritis in rats.¹¹ Tramadol hydrochloride is a centrally acting opioid analgesic, the efficacy and potency of which is only five to ten times lower than that of morphine. This drug has been used in rats^{12,13} and its effect was similar EA in our study on writhing test. The effect of tramadol could be reduced with yohimbine as well as naloxone. The efficacy of tramadol depends in part on the duration of nerve injury-evoked nociception, and that its antinociceptive mechanism changes over time.¹³

We demonstrated that EA significantly increased serum cortisol level even than tramadol (Figure 1). This finding is similar to Wang's et al (2007) study in rats;¹⁴ but is different to some studies e.g. Radahmadi et al (2006) seen a significant increase in serum cortisol level in diabetes rats but stress did not cause significant increases in its level.¹⁵ In some studies, the plasma and serum level of cortisol was measured in rats for visceral dysfunction and inflammation.^{16,17} But it was reported a decrease in plasma cortisol concentration in human

with EA.¹⁸ The mean of serum cortisol increased with tramadol administration with comparison to control group in our study. However, administration of tramadol in dog did not change plasma cortisol concentration.¹⁹

Since corticosteron is major corticosteroid in rats and the precursor of corticosteron and cortisol is same, thus the amount of cortisol is decreased by elevation of corticosteron synthesis during stress and pain.

In conclusion, present study shows that tramadol and EA has similar analgesic effect on writhing test in rats.

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References

1. Yang J, Yang Y, Chen JM, Liu WY, Wang CH, Lin BC. Effect of oxytocin on acupuncture analgesia in the rat. *Neuropeptides* 2007;41:285-92.
2. Koo ST, Lim KS, Chung K, Ju H, Chung JM. Electroacupuncture-induced analgesia in a rat model of ankle sprain pain is mediated by spinal alpha-adrenoceptors. *Pain* 2008;135(1-2):11-9.
3. Cui KM, Li WM, Gao X, Chung K, Chung J M, Wu GC. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. *Neurosci lett* 2005;376:20-3.
4. Fleckenstein J, Kramer S, Hoffrogge P, Thoma S, Lang PM, Lehmeier L, et al. Acupuncture in acute herpes zoster pain therapy (ACUZoster) - design and protocol of a randomised controlled trial. *BMC Complement Altern Med* 2009;12:9:31.
5. Yin CS, Jeong HS, Park HJ, Baik Y, Yoon MH, Choi CB, et al. A proposed transpositional acupoint System in a mouse and rat model. *Res Vet sci* 2008;84:159-65.
6. Grond S, Sablotzki A. Clinical pharmacology of Tramadol. *Clin pharmacokinet* 2004;43(13):879-923.
7. Ghia JE, Crenner F, Metz-Boutigue MH, Aunis D, Angel F. Effects of a chromogranin-derived peptide (CgA 47-66) in the writhing nociceptive response induced by acetic acid in rats. *Regul Peptides* 2004;119(3):199-207.
8. Zanoori A, Tamaddonfard E, Mojtahedin A. Effects of chlorpheniramine and ranitidine on the visceral nociception induced by acetic acid in rats: role of opioid system. *Pak J Biol Sci* 2008;15;11(20):2428-32.
9. Huang C, Hu ZP, Long H, Shi YS, Han JS, Wan Y. Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain. *Brain Res Bull* 2004;63:99-103.
10. Senna-Fernandes V, França DL, de Souza D, Santos KC, Sousa RS, Manoel CV, et al. Acupuncture at zusanli (st.36) and Sanyinjiao (sp.6) Points on the Gastrointestinal tract: A Study of the Bioavailability of Tc-Sodium Pertechnetate in rats. *Evid Compl Altern Med* 2009;1-6.
11. Fang JQ, Shao XM, Ma GZ. Effect of electroacupuncture at "Zusanli" (ST 36) and "Sanyinjiao" (SP 6) on collagen-induced arthritis and secretory function of knee-joint synoviocytes in rats. *Zhen Ci Yan Jiu* 2009; 34(2):93-6.
12. Guzman-Silva MA, Pollastri CE, Pantaleão JAS, de Carvalho ACB, Henriques HN, Camara NR, et al. Tramadol minimizes potential pain during post-oophorectomy in Wistar rats. *Altern Anim Exp* 2007;14:91-2.
13. Hama A, Sagen J. Altered antinociceptive efficacy of tramadol over time in rats with painful peripheral neuropathy. *Eur J Pharmacol* 2007;559(1):32-7.
14. Wang X, Teng JR, Lu B, Jin ZG. Effect of acupuncture of "Zusanli" (ST- 36) on serum gastrin and cortisol contents in rats with spleen deficiency syndrome. *Zhen Ci Yan Jiu* 2007;32(2):125-7.
15. Radahmadi M, Shadan F, Karimian SM, Sadr SS, Nasimi A. Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weight in rats. *Pathophysiology* 2006;13(1):51-5.
16. Gschossmann JM, Buenger L, Adam B, Liebrechts T, Saller B, Mann K, et al. Diurnal variation of abdominal motor responses to colorectal distension and plasma cortisol levels in rats. *Neurogastroenterol Motil* 2001;13(6):585-9.
17. Prasad A, Naskar R, Dubey R, Raha D, Ahmed MF. Modulation of serum cortisol by substance P in albino rats: evidence of a direct effect on adrenal gland. *Indian J Exp Biol* 2006;44(2):163-4.
18. Harbach H, Moll B, Boedeker RH, Vigelius-Rauch U, Otto H, Muehling J, et al. Minimal immunoreactive plasma beta-endorphin and decrease of cortisol at standard analgesia or different acupuncture techniques. *Eur J Anaesthesiol* 2007;24(4):370-6.
19. Martins TL, Kahvegian MA, Noel-Morgan J, Leon-Román MA, Otsuki DA, Fantoni DT. Comparison of the effects of tramadol, codeine, and ketoprofen alone or in combination on postoperative pain and on concentrations of blood glucose, serum cortisol, and serum interleukin-6 in dogs undergoing maxillectomy or mandibulectomy. *Am J Vet Res* 2010;71(9):1019-26.