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Original article

Effect of electroacupuncture at low frequencies on the *Yintang* (extra) and *Baihui* (GV20) points in a murine model with induced depression

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ABSTRACT

Objective: To evaluate the effect of electroacupuncture at low frequencies in a murine model using a modified depression model with the forced swimming test for seven weeks.
Material and methods: 12 mice of the CD-1 strain, two groups ($n=6$), a group with electroacupuncture (EA) treatment (4–6 Hz, 14 sessions of 20 min, for seven weeks) and a group of sham acupuncture (Sham), using a modified depression model, with the forced swim test.
Results and conclusions: In this study, in the EA group a significant effect on depression was shown using the forced swim test. It was determined that the group with EA increased their body weight (on average by 4 g), which could be interpreted as a behavior that seeks to preserve life and therefore mice retain their appetite toward achieving adequate growth and development. In this way, it can be shown that EA has an antidepressant effect, favoring increased motivation to survive and reducing behaviors corresponding to states of depression.

Efecto de la electroacupuntura a bajas frecuencias en los puntos *Yintang* (extra) y *Baihui* (GV20) en un modelo murino con depresión inducida

Palabras clave:
Depresión
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4 Hz
Baihui y *Yintang*

RESUMEN

Objetivo: Evaluar el efecto de la electroacupuntura a bajas frecuencias en un modelo murino, utilizando un modelo de depresión modificada con la prueba de natación forzada, durante 7 semanas.
Material y métodos: Un total de 12 ratones de la cepa CD-1 divididos en dos grupos ($n=6$): un grupo con tratamiento de electroacupuntura (EA) (4-6 Hz, 14 sesiones de 20 min, durante 7 semanas) y un grupo de acupuntura sham (Sham), utilizando un modelo de depresión modificada, con la prueba de natación forzada.

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Resultados y conclusiones: En este estudio, en el grupo EA, se observó un efecto significativo en la depresión, utilizando la prueba de natación forzada. Se determinó que el grupo EA incrementó su peso corporal (en 4 g, de media), lo cual podría interpretarse como un comportamiento que persigue preservar la vida y, por tanto, los ratones retienen su apetito hasta lograr un crecimiento y desarrollo adecuados. De este modo, puede observarse que EA tiene un efecto antidepresivo, favoreciendo la motivación para sobrevivir y reduciendo los comportamientos correspondientes a los estados de depresión.

Introduction

Acupuncture is used to treat many diseases, is needed to explain its mechanism of action. In this study we demonstrate the effects of electroacupuncture (EA) at low frequencies (2–4 Hz) in a murine model with a modified model of depression. Depression is one of the most frequent mental disorders in the world, representing the main cause of disability; it is estimated that it affects approximately more than 300 million people in the world, with the female sex being the most affected by this pathology.[1] To better understand depression and in search of treatments for it, studies have been carried out in animal models (mainly rats and mice). There are two types of depression, reactive and endogenous, the reactive is the most used in this type of model; which are based on exposing the individual to environmental situations for which he has no alternative solution, evaluating his response.[2]

One of the models where hopelessness is induced and with which behaviors that indicate a state of depression in animals can be evaluated, is the forced swimming model; where it is possible to observe immobility, which indicates an abandonment of the motivation to escape, representing loss of interest to his life.[2,3]

In previous studies it has been shown that EA has an anxiolytic effect,[4] also that it favors the decrease of the neurotransmitter acetylcholine, helping in the inflammatory process, promotes contact between cells and favors the start of the repair process,[5,6] acupuncture in a mouse model with stress and lack of sleep has also been shown to generate effects on inflammatory markers (adenosine receptors MMP-9 and A2A).[7]

Other studies have shown the importance of the serotonergic system in depressive disorders. In rats, using *Zusanli* (ST36) and *Sanyinjiao* (SP6) with the forced swimming test,[8] the rats were individually forced to swim 15 min in a vertical plexiglass cylinder containing 15 cm of water. They were placed back into the cylinders 24 h later and the total duration of immobility was measured during a 5 min test,[9] in which with the application of acupuncture, the mice/rats have greater mobility during the test; this shows that they have less depression data generated by this test. There are works that use other points, *Baihui* (GV20) and *Yintang* (extra) for depression, also with a favorable effect[4]; applying the EA at low frequencies (frequency of 2 Hz and an intensity of 0.6 mA for 30 min/day).[4] Tests were performed where EA was applied at 2 Hz in *Yintang* (extra) (anterior midline) and *Baihui* (GV20) (posterior midline) in the head of rats for three days or four days, reporting that at three days of EA they did not modify the behavior of the rats in the forced swimming test; however, at five days of treatment, the rats showed significant changes, showing longer escalation times and shorter periods of immobility during the forced swimming test. Proposing that EA at 2 Hz induces the release of β-endorphins and enkephalins in the brain, which may have anxiolytic and analgesic effects under stress conditions.[4]

An important neurotransmitter in anxiety-depression is serotonin (5-HT), it is one of the goals to regulate through acupuncture. The mechanisms activated by EA can differ according to the stimulation frequency; low-frequency EA (<15 Hz) increases spinal release of met-enkephalins, endomorphins, and β-endorphins, this has been demonstrated even with anesthetized rats.[10] As reported, spinal serotonergic modulation is involved in the production of antinociception by 2, 100 and 2/100 Hz of EA, influencing the availability of serotonin in the spinal cord.[10]

Another important effect of acupuncture is the modification of the viscosity of the blood, which increases as a result of stress, and with the application of acupuncture mechanisms that affect levels of cytokines, hormones and leukocytes are generated. The effect on lipid profiles such as low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides has also been reported to affect blood viscosity,[11] which may be related to some risk factor such as overweight and obesity, as well as the consequences that will be generated with these blood components.

Materials and methods

Animals

They were used 12 males mice (6 per group) of the strain CD-1, from 8 to 10 weeks old with a weight between 30 and 34 g, in all groups we used standard diet (Rodent Laboratory Chow 5001-Purina) and water, at an ambient temperature of 22 °C with light/dark cycles of 12 h. Were formed two groups of 6 mice each one of them, a Sham group (acupuncture simulated). The management of the animals was complying with the accommodation and feeding procedures established by the postgraduate bioethics committee and complying with the norm of handling and use of laboratory animals NOM-062 (Official Mexican Standard NOM-062-ZOO-1999).

Table 1 – Average body weight in grams of the Sham and electroacupuncture group with the standard deviation ($n = 6$).

Week	Sham	SD	*Baihui* and *Yintang*	SD
Initial	31.83	1.4	32.5	1.5
1	31.8	1.1	32.5	2.0
2	31.1	0.7	32.6	1.7
3	31.8	1.1	34.3	2.4
4	31.6	1.2	34.3	2.6
5	32.3	1.2	35.3	3.2
6	32.3	1.1	35.3	2.8
7	32.3	1.0	36.5	2.8

Electroacupuncture (EA)

The procedures were performed with mice lightly anesthetized with "Sural" (0.1 mg/kg), as mentioned above to minimize immobilization-induced stress that needs to be applied to mice for needle insertion and stimulation.[10] Stainless steel acupuncture needles (0.18 × 13 mm Hwato) were used. The electrostimulation equipment brand HWATO model SDZ-II was used for 14 sessions with duration of 20 min each one of them, at frequencies of 4–6 Hz, with continuous frequency wave, applied in *Baihui* (GV20) and *Yintang* (extra) point. Sham acupuncture: the same points and conditions, mice lightly anesthetized with "Sural" (0.1 mg/kg), it is applied pressure of one second with the blunt tip of the needle that stimulates the point without penetrating the skin.

Forced swimming

Based on models previously described,[4,8,9] mice were placed individually in a cylinder containing 20 cm of water at a temperature of 25 °C, ensuring that they cannot stand on the bottom, forcing them to swim for 15 min, recording the behavior of the mice, mainly indicators of hopelessness (immobility).[8,9] We modify the sessions, in which we perform forced swim sessions for 15 min, twice a week, for 7 weeks, in total 14 sessions. Evaluating the mobility of each swim test and their body weight.

Statistical analysis

Averages and standard deviations were calculated in Excel (Microsoft). Student's *t*-test, and *P* values will be calculated using Graph Pad software. To designate whether comparisons between two sample groups have statistical significance, a $P < 0.05$ value will be deemed significant.

Results

When evaluating the data of the forced swimming test (with the 7-week model of depression), the mice with EA showed a decrease in the immobility time and an increase in the period of escalation during the forced swimming test, also finding an increase in body weight over seven weeks. Mice from the CD-1 strain of the EA group (*Baihui* (GV20) and *Yintang* (extra)) showed significant weight gain at 7th week (Table 1), the animals in the Sham group did not gain weight significantly. Fig. 1

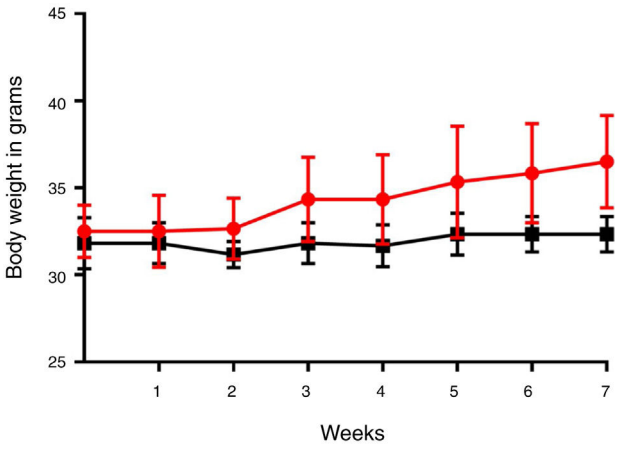

Fig. 1 – Body weight in of mice group per week. Black: Sham group, red: *Baihui* (GV20) and *Yintang* (extra) group ($n = 6$ mice in each group). Data are presented as mean ± standard deviation of mean. There is a statistical difference between both groups in the 7th week, determined by *t* test ($P = 0.0012$).

shows the average body weight (BW) of each group weekly. The data was analyzed with the Graph Pad software. It was determined that at the end of 7th week, all the mice with EA, fed with the standard diet, presented the same growth trend and the average of the BW of 36.5 ± 2.8 g., While the Sham group fed with the standard diet, the average of the BW of 32.3 ± 1.0 g. After seven weeks of treatment with EA, there is statistically significant differences ($P = 0.0012$) were observed in the growth curves of each experimental group.

During the seven weeks, an increase in the escalation period was observed in the group with EA during the forced swimming test, as well as a decrease in the immobility time, which represents according to previously described models,[3,9] an improvement in behaviors equivalent to a state of depression; showing an increased interest in fighting and preserving life. Compared with the Sham group, where during the forced swimming tests the immobility time was increasing and the escalation period was reduced, reaching in the last weeks, an almost immobility in the forced swimming test.

Discussion

In this study, the effect of electroacupuncture was determined, using a modified depression model, which we evaluated and compared with the results of other works.[8,9] Studies have been reported where they have extended treatments to five days with important results in the regulation of the organism.[4] We evaluated the effect at seven weeks using the *Baihui* (GV20) and *Yintang* (extra) points, which have shown an important effect on depression.[4] Neurobiological studies of EA at these points have shown that electrical stimulation at 2 Hz alters the signaling of the N-methyl-D-aspartate receptor (NMDA), favors a greater expression of the brain-derived neutrootrophic factor (BDNF) and the receptor for tropomyosin kinase (TrkB), predominantly in the hippocampus; it is one of the areas significantly involved in the stress response.[4] This reinforces the

explanation of some of the mechanisms of action of EA, favoring the antidepressant effects in this model.

So, we also sought to evaluate the relationship between depression and body weight, which is why we extended the forced swimming tests, which was very interesting to observe that, in the 7th week, we obtained a significant result ($P = 0.0012$), with an average increase of 4 g in the group with EA. To be able to relate the data shown by the forced swimming test[2,3] and acupuncture is a big challenge, since there are reports that demonstrate how with EA in different points such as *Zusanli* (ST36), *Sanyinjiao* (SJ6), *Baihui* (GV20), *Yintang* (extra), among others, the secretion of different neurotransmitters, peptide hormones or messengers that have to do with the regulation of an altered process can be regulated. As well as relating the use of low frequency EA and the effect on the regulation of serotonin, beta-endorphins and acetylcholine, seeking to justify its effect on depression[6,8,10] and how these may be participating in body weight gain in the EA group in this study.

We propose that EA helps to decrease behaviors related to a state of depression and, in turn, these have an effect on body weight gain. This may represent an improvement in the motivation to preserve life, favoring the mice to have an adequate food intake, compared to the Sham group, where the state of depression was observed and the behaviors of hopelessness and loss of interest in the life became generalized, leading the animals to decrease food intake and difficulty increasing their body weight. Furthermore, we can relate the effect of EA on blood homeostasis, since it has been shown that when using EA in *Zusanli* (ST36), better values are seen in the blood components (triglycerides, cholesterol and fibrinogen),[11] this being an indication that applying acupuncture favors the regulation of metabolism, which leads to homeostasis, giving greater sustenance by promoting adequate development with an optimal weight increase in mice, to have a favorable assimilation of nutrients.

Conclusion

EA was shown to have a significant effect on depression in this study. Since, in the murine model, with the modified forced swimming test, a state of depression was perpetuated, and with the application of electroacupuncture at low frequencies it was possible to observe a decrease in signs of hopeless, characteristics of depressive states, finding modification in immobility times; which decreased in the EA group compared to the Sham group. Also finding an increase in the escalation period in the EA group, the effect in the Sham group being contrary. Another important aspect observed in this study is the modification of body weight, which increased in the group with EA (on average 4 g), Which could be interpreted as a behavior that seeks to preserve life and therefore mice conserve their appetite, having adequate growth and development.

In this way, it can be shown that the EA has an antidepressant effect, favoring the decrease in the lack of motivation to survive and reducing behaviors corresponding to states of depression. With which we can conclude that this therapeutic method within acupuncture has great utility for the treatment of depression, demonstrating important changes in the behavior and body weight of this model, thus showing the effectiveness of EA at low frequencies.

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Conflict of interests

The authors declare that they have no conflict of interests.

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