
Pain modulation in patients with fibromyalgia undergoing acupuncture treatment is associated with fluctuations in serum neuropeptide Y levels

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ABSTRACT

Objective. Neuropeptide Y (NPY) is a neurotransmitter released by sympathetic neurons, which is probably involved in pain modulation. Acupuncture is increasingly used as an alternative or complementary means of controlling pain in rheumatic diseases such as fibromyalgia (FM), a chronic widespread pain syndrome accompanied by allodynia and hyperalgesia. The aim of the present study was to assess the effects of an acupuncture cycle on serum NPY levels in patients with FM, and identify possible correlations between its serum levels and clinical and clinimetric parameters.

Methods. The study involved 30 FM patients who underwent clinical and clinimetric evaluations and blood sampling at baseline and at the end of the treatment, and 20 healthy subjects who underwent blood sampling.

Results. The baseline serum NPY levels of the patients were higher than those of the controls. They had significantly increased by the end of the treatment, when there was also a statistically significant reduction in pain, the number of tender points number, and the clinimetric scores.

Conclusion. These findings confirm the analgesic properties of acupuncture as a complementary treatment in FM, and indicate that NPY could play a role in pain modulation.

Introduction

Fibromyalgia (FM) is a chronic syndrome characterised by widespread and lasting pain, accompanied by hyperalgesia and allodynia (1). Its pathogenesis is not fully known but involves psychological, genetic, neurobiological and environmental factors. One of the main pathophysiological bases of

the disease is the mechanism of central sensitisation, which is considered to be an expression of neuronal plasticity and is likely to amplify pain perception. The role of other mechanisms still has to be clarified (2), but autonomic nervous system dysfunction may partially explain some of the multi-systemic features of FM that are due to a sympathetic/parasympathetic imbalance (3, 4); other symptoms include debilitating fatigue, non-restorative sleep, joint stiffness, cognitive dysfunction and mood disorders (5-8). FM is frequently comorbid with psychiatric conditions such as depression and anxiety, and stress-related manifestations such as post-traumatic stress disorder.

The best approach to FM treatment combines pharmacological and non-pharmacological interventions (8, 10). Acupuncture, a technique used in traditional Chinese medicine (TCM) that is based on the insertion of very thin needles into subcutaneous tissue, is increasingly being used as an alternative or complementary pain therapy (11). According to the principles of TCM, the typical symptoms of FM are caused by an imbalance in the flow of blood and/or internal energy (*qi*) (12-14). There are two types of acupuncture: conventional or somatic acupuncture, which is based on acupoints distributed throughout the body, whereas the method recently introduced by Dr Bo Yun Zi uses a microsystem of acupoints restricted to the peri-umbilical zone, and seems to be very effective in promptly reducing pain. However, although acupuncture is an ancient treatment, the chemical responsible for its analgesic action is still unknown.

Neuropeptide Y (NPY), a highly conserved neuropeptide, reduces anxiety and stress (15), and also has an analge-

Competing interests: none declared.

sis function. Jin-Ju Li *et al.* (16) have investigated its role in nociceptive modulation in rats: NPY receptors are distributed abundantly in the arcuate nucleus of rat hypothalamus (ARC), a structure involved in pain processing, and they found that inoculating NPY in the ARC induced dose-dependent antinociception, whereas the intra-ARC injection of NPY antiserum or NPY receptor antagonist enhanced the hyperalgesia (16).

In order to study the anti-nociceptive role of NPY in humans, we recently investigated its serum concentrations in FM patients, and found that they were higher than those measured in two control groups of healthy subjects and patients with systemic sclerosis (17).

The aim of this study was to assess the effects of a cycle of acupuncture on serum NPY levels in women with FM, and verify the possible relationship between NPY levels and clinical and clinimetric scores before and after acupuncture treatment.

Materials and methods

Patients

We consecutively enrolled female patients referred to the Rheumatology Unit of the Sapienza University in Rome, Italy, and diagnosed as having FM on the basis of both the 1990 and 2010 American College of Rheumatism (ACR) criteria (1, 5). In order to enter the study, they had to have received the same treatment for at least three months or not to have received any treatment.

All of the patients signed an informed consent form, and underwent a cycle of 10 weekly acupuncture sessions. At baseline (T0), and after five (T1) and ten weeks (T2), they also underwent a clinical examination, including a tender point (TP) count and clinimetric evaluations.

Blood samples were taken five minutes before the first session, five minutes before and after the fifth session, and five minutes after the last session, and the serum was stored at a temperature of -20°C until tested. In order to compare baseline NPY levels, 20 age-matched female healthy subjects were also evaluated as a control group.

The study was approved by our local Ethics Committee (Sapienza University - Policlinico Umberto I, Rome, Italy).

Clinimetric evaluations

In order to evaluate their symptoms and the impact of FM on their quality of life, the enrolled patients completed the Fibromyalgia Impact Questionnaire (FIQ), the Fibromyalgia Assessment Status (FAS), the Health Assessment Questionnaire (HAQ), pain and disease activity visual analogue Scales (VAS), and the Zung Self-Rating Anxiety and Depression Scales (ZSAS, ZSDS) (18-22).

Acupuncture

An expert physician (FM) diagnosed all of the patients in accordance with the principles of TCM and then selected the appropriate acupoints for the 10 weekly somatic and abdominal acupuncture sessions. Before each session, the physician re-evaluated the patient to determine whether the clinical situation had changed and, if so, the selected acupoints was reconsidered (23). Every session took place in a restful room using size 0.18x30 mm and 0.18x40 mm sterilised, single-use stainless steel filiform needles (Hwato-Suzhou Medical Appliance Factory, Suzhou, China), with the patient lying in a supine or prone position.

In the case of somatic acupuncture treatment, unless otherwise indicated, the needles were inserted vertically to a predetermined depth (normally 8–20 mm) depending on the location of the point, which was then stimulated by bidirectionally rotating the needle sleeve until the patient reported the sensation known as *de-qi*, a sort of dysesthesia that is commonly described as a “glowing” feeling and represents “the flowing of *qi*” (21). Depending on the TCM diagnosis, the physician inserted the needles in a variable number of 23 DU, Yn Tang (extra), four large intestine (LI), 36 stomach (St), six spleen (SP), seven lung (L), three kidney (K), three liver (LR), three small intestine (SI), 62 urinary bladder (UB), six pericardium (Pc), and six San Jiao (SJ) acupoints (21), and the needles were kept in place for 20 minutes before being removed.

In the case of abdominal acupuncture treatment, the needles were simply in-

serted in the abdominal skin without any rotation or movement. Depending on the TCM diagnosis, the selected abdominal acupoints were 12 and four Ren, 25 St bilaterally, 15 SP bilaterally, and 24 St points bilaterally. Nine Ren and extra points were used for pain located above the diaphragm, and 26 St bilaterally, 11 and 17 kidney (K) and extra points were used for pain located in the lower part of the diaphragm (24). A record was made of all of the acupoints and techniques used.

NPY ELISA

Serum NPY levels were measured by means of an enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (Phoenix Europe GmbH, Karlsruhe, Germany) as previously described (25) and the results were expressed as ng/mL. Serum NPY levels were evaluated in the FM patient before the acupuncture sessions at T0, T1 and T2 in FM patients and, in order to evaluate rapid fluctuations further, also five minutes after the session at T1.

Serum NPY levels were also evaluated in 20 age- and gender-matched healthy donors as control group at baseline.

Statistical analysis

All of the clinical and laboratory data were collected in standardised electronic case report forms for statistical evaluation. Normally distributed variables were summarised using mean values \pm standard deviation (SD), and non-normally distributed variables were summarised as median and ranges. Univariate comparisons between the nominal variables were made using the chi-squared test or Fisher's exact test as appropriate. Differences in the number of TPs and clinimetric scores, and between-group differences in laboratory values were analysed using the Mann-Whitney U-test (MW). Matched pairs were analysed using Wilcoxon's signed rank test (WT). A *p*-value <0.05 was considered statistically significant. All of the analyses were made using v. 13.0 of the SPSS statistical package.

Results

The 30 consecutively enrolled women

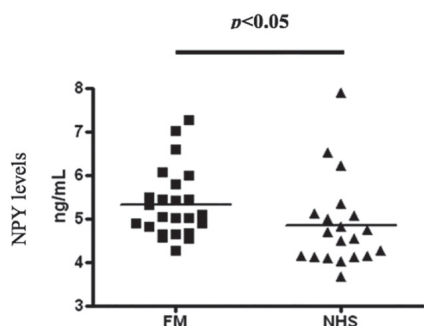


Fig. 1. Baseline serum NPY levels in the FM patients and in the age- and gender-matched healthy subjects. NHS: normal human serum.

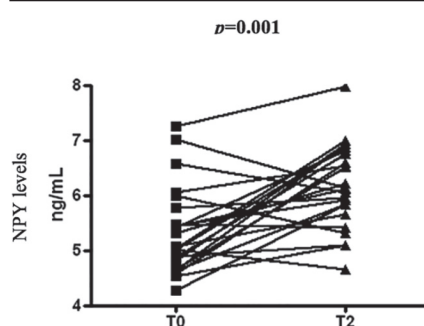


Fig. 2. Post-treatment changes in serum NPY levels.

with FM had a median age of 50 years (range 26-67yrs), and a median disease duration of 60 months (range 13-240). Six patients dropped out (two because they were not responsive to treatment and four because of poor compliance). At the time of enrolment, nine of the 24 analysed patients were not taking any medication, and 15 had been on stable drug therapy for the previous three months (four on selective serotonin reuptake inhibitors (SSRIs), four on amitriptyline, four on muscle relaxants, four on micronutrient supplementation, three on the anti-epileptic drug pregabalin, and one on tramadol). All of these patients completed the planned course of acupuncture without taking any additional medications.

Baseline serum NPY levels were higher in the FM patients than in the healthy subjects (mean \pm SD: 5.05 ± 0.78 vs. 4.61 ± 1.02 ng/mL; $p=0.01$) (Fig. 1).

The analysis of NPY levels before and after the fifth acupuncture session (T1) showed a non-significant increase from 4.88 ± 0.97 ng/mL to 5.25 ± 0.72 ng/mL ($p=0.09$), while a statistically significant increase was observed after 10 acupuncture sessions (T2) compared

Table I. Clinical and clinimetric data and NPY levels at baseline and after acupuncture treatment.

	T0 median (range)	T2 median (range)	<i>p</i> -value
No. of TPs	17 (13-18)	13 (5-18)	0.0001
Pain VAS	80 (50-100)	50 (0-100)	0.0001
FIQ	67.6 (62.2-91.2)	47.6 (3.6-69.4)	<0.0001
FAS	8 (6.43-9.8)	6.53 (4.5-9.6)	0.006
HAQ	1.43 (0.37-2.12)	0.87 (0.12-1.87)	0.002
Disease activity VAS	77.5 (50-100)	50 (0-95)	0.0001
ZSAS	52.5 (37-80)	49.5 (33-71)	0.0002
ZSDS	52 (36-74)	48 (31-68)	0.002
NPY levels, ng/mL*	5.05 ± 0.78	6.12 ± 0.76	0.001

*expressed as mean \pm standard deviation (SD).

TPs: tender points; FIQ: Fibromyalgia Impact Questionnaire; FAS: Fibromyalgia Assessment Status; HAQ: Health Assessment Questionnaire; VAS: visual analogue Scales; ZSAS and ZSDS: Zung Self-Rating Anxiety and Depression Scales.

to the baseline levels (mean \pm SD, T0 5.05 ± 0.78 ng/mL vs. T2 6.12 ± 0.76 ng/mL $p=0.001$) (Fig. 2).

There was also a statistically significant reduction in the number of TPs, pain and disease activity VAS ($p=0.0001$; $p=0.0001$), FIQ ($p<0.0001$), FAS ($p=0.006$), HAQ ($p=0.002$), ZSAS ($p=0.0002$) and ZSDS scores ($p=0.002$) from T0 to T2.

Table I shows the clinical and clinimetric data and NPY levels at baseline and after acupuncture treatment.

There were no correlations between the change in NPY levels and the number of TPs or the scores for pain, fatigue, sleep disturbances, morning stiffness, anxiety and depression, state of well-being or the quality of life.

Discussion

The results of this study show a significant increase in serum NPY levels in a group of FM patients undergoing 10 sessions of acupuncture treatment, and a significant improvement in clinical variables by the end of the acupuncture cycle.

Serum NPY is one of the possible chemical mediators of the therapeutic activity of acupuncture in FM patients and, to the best of our knowledge, ours is the first study to analyse the changes in NPY levels in acupuncture-treated patients. In order to avoid confounding therapeutic factors, the study included patients not taking any medication or those that had been on stable therapy for at least three months.

In accordance with other authors (17,

26, 27), we found that FM patients had higher baseline serum NPY levels than the healthy controls ($p=0.01$). In addition, there was a statistically significant increase in serum NPY levels after the full cycle of acupuncture treatment ($p=0.001$), and the fact that the levels in the sample taken after the fifth acupuncture session were higher than those in the sample taken before it indicates the existence of short-term variations. This difference was not statistically significant, but it was accompanied by a partial improvement in symptoms that may act as positive reinforcement for the patients and at least partially explain their generally good compliance with the treatment protocol.

At the end of the ten somatic and abdominal acupuncture sessions, there was a statistically significant reduction in the number of TPs and in the pain and disease activity VAS, FIQ, FAS, HAQ, ZSAS and ZSDS, thus indicating an improvement in all of the key aspects of FM.

Anderberg *et al.* (28) found increased NPY levels in patients with FM or other conditions characterised by chronic pain, and hypothesised that long-lasting pain may activate the anxiolytic and sedative effects of NPY. The same authors have also observed an increase in peripheral blood NPY levels in a group of patients with pain caused by burns, and other authors have described an increase in NPY levels in patients with chronic pain syndromes when the pain decreased and *vice versa* (29, 30). NPY and its receptors

also seem to be part of a mechanism whereby mammals naturally recover from hyperalgesia (31), which is one of the central clinical characteristics of FM, and the fact that there is a close relationship between stressors and the onset of FM has been demonstrated in a model of chronic widespread pain induced using intermittent sound stress in rats (32). Consequently, although the exact mechanism is still unclear, it can be speculated that the increase in NPY levels in painful conditions is an initial attempt to minimise pain.

Acupuncture seems to affect the balance between the endocrine, immune and central nervous systems as a result of the release of various chemical mediators into the blood stream (33). In general, many diseases are associated with alterations in chemical mediators such as substance P, enkephalin, beta-endorphins, NPY, TNF, and interleukins 1 and 6, and acupuncture treatment normalises their blood levels (34).

A number of authors have studied the effects of acupuncture on NPY levels with conflicting results, but all of these studies used rats (35-37). The most intriguing possible explanation for the high baseline levels and the fluctuations observed in our FM patients (as a whole and individually) in response to the acupuncture treatment, is that it represents the attempts by the neuroimmune system to react to stressor-like pain. In this hypothesis, as previously described in the case of soldiers subjected to great psychological stress (38, 39), NPY seems to be a protective factor capable of mitigating the consequences of stress exposure. Soldiers show significantly higher NPY levels when exposed to combat (38) and, subsequently, express greater feelings of dominance and confidence associated with superior performance during stress (39). It is therefore possible to speculate that the pain reduction observed in our FM patients was the result of stress containment.

This is the second study investigating the effect of combined somatic and abdominal acupuncture on FM patients (40). The reason for using both is that, in our experience, the abdominal technique is very effective in promptly re-

ducing pain, does not require needle manipulation, and is well tolerated by patients because it acts remotely and thus avoids the typical symptom exacerbation caused by direct manipulation, whereas somatic acupuncture is systemic and suitable for treating the signs and symptoms associated with FM. The combination of the two methods makes it possible to achieve pain control and improve the quality of life, thus giving it the potential to reduce drug abuse and physician consultations and, consequently, the social costs of the disease. Nevertheless, a properly designed trial is needed to clarify the specific effects of the two techniques, compare their efficacy and tolerability, and evaluate the usefulness of their combination.

Our study is informative concerning the potential role of NPY in pain perception and modulation of FM, and the potential role of acupuncture in treating FM, both of which support the value of carrying out a properly designed clinical trial.

However, our study presents some limitations. The first one is the small number of patients, which, although justified by the fact that this was a pilot study, may have biased the results. The small sample size may also explain why no correlation was found between NPY levels and the clinical improvement.

We also had a relatively high drop-out rate (6/30 patients, 20%). Two patients dropped out in the second week of treatment because of a lack of response, and four because of poor compliance (one in the second week, one in the third, and two in the fourth). Compliance is an essential aspect of acupuncture treatment because of the number and duration of individual sessions, and its effectiveness can only be assessed at the end of an entire cycle, as it is based only on reiterated sessions. The risk of drop-outs, together with the interference of fear (with the related stress response) during the first session, and other possible disturbances, led us to investigate the immediate effects of a single session by measuring NPY levels before and after the fifth session, when the treatment had stabilised and

confounding factors had at least been minimised.

Another limitation is that NPY was the only neurotransmitter measured, and we did not consider other potential mediators of pain. However, we decided to investigate NPY alone because it has the great advantage of being more stable and have a longer serum half-life than other neurotransmitters (*e.g.* norepinephrine), and there was previous evidence of its potential role in the pathophysiology of FM (41). Future studies should investigate possible variations in other chemical mediators in order to identify the neurotransmitters involved in the acupuncture treatment of FM patients.

Conclusions

Acupuncture is a complementary and sometimes alternative means of treating pain (42). The American College of Physicians and the American Pain Society have stated that it can be considered as a possible treatment option for patients with chronic low back pain who do not respond to self-care (43, 44).

This non-pharmacological approach, being used by an increasing number of FM patients, is becoming a cornerstone of FM treatment (45) and has been recently considered also in the revised EULAR recommendations for managing FM (46). Its exact mechanism of action is still unclear, but the fluctuating serum NPY levels associated with acupuncture provides biochemical basis for its effectiveness and may at least partially explain the pain relief obtained by FM patients. Furthermore, although it is not always easy to carry out, it is often also effective in reducing other FM symptoms.

In conclusion, the results of this study increase our understanding of the complex role of NPY in pain modulation and open up the possibility of new therapeutic approaches. It also confirms the analgesic properties of combined acupuncture, and its potential role in reducing both the drug use and the social costs of FM (47).

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