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Research Article

Acupuncture decreases pain and MIF salivary levels in men and women

Abstract

Acupuncture is used worldwide to treat many diseases, including painful conditions. Acupuncture-induced effects on pain were studied in men and women to understand the various factors involved in this positive procedure able to cure patients without side effects. Vital parameters (heart rate, blood pressure, temperature) were measured and questionnaires about quality of life (SF-36), mood state (POMS) and pain condition (QUID) were administered to men and women requesting acupuncture treatment for pain. Macrophage inhibitory factor (MIF) was determined in the saliva. All parameters were determined before the first and tenth acupuncture sessions. Pain scores (questionnaire and scales) significantly decreased from the first to the last acupuncture session in both sexes. Vital parameters were not affected, while the other questionnaires indicated a general improvement in the quality of life. MIF salivary levels were higher in males than in females and were significantly decreased by acupuncture in both sexes.

Conclusion: In conclusion, acupuncture appears to affect pain also by decreasing important cytokines such as MIF.

Keywords: acupuncture, MIF, gender differences, pain, pain assessment

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Abbreviations: BMI, body mass index; MIF, macrophage inhibitory factor; POMS, profile of mood state; SF-36, short form-36; QUID, italian pain questionnaire; VAS, visual analogue scale; PRIr-T, pain rating index rank value; PPI, present pain intensity, HR, heart rate, BP, blood pressure

Introduction

Acupuncture has been part of mainstream medicine in China for thousands of years and has made great contributions to the health and health care of the Chinese nation; it was spread to Europe and the Americas from the sixteenth to the nineteenth century.[1] Acupuncture needles, either manipulated manually or stimulated using a low current and frequency, have been found to modulate the activity of peripheral and central neural pathways.[2] The effectiveness of acupuncture has been documented in areas such as relieving neck and back pain, migraines and headaches, and knee osteoarthritis symptoms.[3] Several mechanisms have been suggested to explain its efficacy, including a number of neural circuits and their transmitters and modulators such as beta-endorphin, serotonin, substance P, interleukin and calcitonin gene-related peptide.[4]

Macrophage migration inhibitory factor (MIF), one of the first cytokines to be identified, is a soluble protein produced by T-lymphocytes with the ability to inhibit the random migration of macrophages.[5] Knowledge about MIF has been expanded to include its role in a variety of pathophysiological states mainly based on acute and chronic inflammatory disorders.[6] Consistent with its role in inflammation, MIF is involved in the pathogenesis of different diseases such as systemic infections and sepsis, autoimmune diseases, cancer and metabolic disorders.[6] Each of these conditions is characterized by high levels of serum MIF, a feature for which MIF is regarded as a biomarker of many autoimmune and inflammatory diseases.[7] Studies in various animal models have shown that inhibition of MIF produces beneficial effects in many diseases such as severe sepsis,[8] rheumatoid arthritis,[9] allergic airway inflammation,[10] colitis,[11] bladder inflammation,[12] chronic obstructive pulmonary disease[13] and acute pancreatitis[14]

A number of studies have demonstrated that MIF is also a regulator for diseases related to chronic pain such as chronic pelvic pain syndrome[15] and interstitial cystitis/bladder pain syndrome (IC/BPS).[12] In an IC/BPS mouse model, Vera et al.[16] showed that inhibition of MIF by treatment with a MIF inhibitor (ISO-1) prevented bladder inflammation and pain. In a later study using MIF$^{-/-}$ mice (referred to as KO mice) in an IC/BPS model, some of the authors of the previous paper along with others showed that MIF mediated bladder pain but not inflammation.[17] Indeed, both wild type (WT) and KO mice showed marked signs of bladder inflammation, while WT but not KO mice developed bladder pain.[17]

Therefore, research on MIF clearly indicates that this cytokine is a key regulator of inflammation and pain and that inhibition of its activity and/or production can improve the condition and progression of disease. Hence, MIF is a potential target for therapeutic modulation of persistent inflammation and pain associated with many diseases.

In this study, we focused on the changes in pain scores, mood state and quality of life parameters, and MIF salivary levels in men and women before and after acupuncture treatment.

Material and methods

Subjects

Male and female outpatients of an acupuncture center in Arezzo (Dr. Fu Bao Tian) were randomly recruited for the study. Inclusion criteria were: presence of pain, willingness to collaborate in the different experimental phases, signing of the informed consent form. Exclusion criteria were: presence of a chronic disease like diabetes, age lower than 18 and higher than 75 years.

Experimental design

Before the first acupuncture session, the patient was asked to fill in questionnaires, to submit to all measurements and to give a saliva sample. All patients were treated according to their primary pathology twice a week for 10 sessions. In particular, after the patient was relaxed, the acupuncturist sterilized the skin around the acupuncture point and then inserted stainless steel needles into the acupuncture points considered significant for the pathology. The patient lay on the bed for 30 min before needles were removed**.** Before the tenth and last acupuncture session, the patient was asked to fill in the questionnaires again, the measurements were repeated and the saliva sample was collected.

Questionnaires

A screening questionnaire asked about age, weight and height, physical activity and cigarette smoking. For patients included in the study, pain intensity was assessed with dedicated questionnaires (VAS, QUID) and other parameters were collected:

a. axillary temperature (left and right arm), blood pressure, heart rate;

b. questionnaires for anxiety and quality of life (POMS, SF-36);

c. saliva sample

VAS: a Visual Analogue Scale, VAS (0-10), was used to estimate the current intensity of pain and the peak intensity during the previous week. VAS was divided into three parts of the day: morning (VAS1), afternoon (VAS2) and night (VAS3).

QUID: The Italian Pain Questionnaire (QUID), a reconstructed Italian version of the McGill Pain Questionnaire, evaluated the quality and intensity of the current pain experience.[18] The questionnaire consists of 42 descriptors divided into 4 pain rating index ranks (sensory, affective, evaluative, mixed). The Total Pain Rating Index rank value (PRIr-T), given by the sum of all the rank values, describes and quantifies pain.

POMS: The Profile of Mood State (POMS)[19] measures the current psychological state of the participant and consists of 58 feelings rated on a 5-point scale. It comprises six subscales: Tension-Anxiety (T-A), Depression-Dejection (D-D), Anger-Hostility (A-H), Vigor-Activity (V-A), Fatigue-Inertia (F-I), and Confusion-Bewilderment (C-B). In each subscale, values higher (T-A, D-D, A-H, F-I, C-B) or lower (V-A) than 55 were considered significantly altered with respect to the normal population.

SF-36: The Italian version of the SF-36 questionnaire is a generic multidimensional instrument for assessing quality of life consisting of 36 items grouped into two components: Physical Component Summary (PCS-36), including physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH); Mental Component Summary (MCS-36), which refers to vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). Individual items are scored on a 0-10 standardized Likert scale. For each domain, including bodily pain, and the component summaries, a higher score indicates a better quality of life and lower limitations. The two component summaries (Physical and Mental) based on pertinent subscale clustering give an overview of the respective functioning.

Saliva collection

Saliva samples were collected using the Salivette collection device (Sarstedt Inc., Nuembrecht, Germany). The subjects took a cotton wool tamponade out of a small tube, placed it in their mouth, chewed on it for 30-45 sec, and then put it back in the tube. Samples were centrifuged and stored at −20 °C until MIF determination.

MIF determination in saliva sample

Saliva samples were assayed for MIF using a colorimetric sandwich enzyme-linked immunosorbent assay (ELISA) protocol developed by Ietta et al. at the University of Siena, Italy.[20] The sensitivity limit of the assay was 18 pg/ml. Intra- and inter-assay coefficients of variation were 3.86 (0.95) and 9.14 (0.47), respectively. The MIF concentration was expressed as pg/ml of saliva.

Statistical analysis

All measures are reported as mean±SEM. ANOVA was applied with the factors Sex (2 levels: male and female) and Test (2 levels: before and after acupuncture treatment). Post hoc analysis was carried out when required. Significance was accepted with $p<0.05$.

Results

Table 1 reports a demographic summary for the 22 outpatients recruited, 8 males and 14 females, including: Sex, Age, Weight, Height, BMI, Activity, Smoking, Pain localization, Acupuncture points.

Pain features and acupoints used

The mean age of patients was 58.1 (range 42-66) for males and 54.3 (range 42-65) for females. Pain was of different origin with most of the patients reporting neuropathic pain (i.e. trigeminal neuralgia, sciatic nerve pain). Patients were treated for their primary pathology. The acupoints are reported in Table 1 and Figure 1 shows four points that are used frequently: GB20, DU24, ST36, BL40.

VAS values were quite similar in the two sexes during Test I, while at Test II the decrease was greater in males than in females in all three measures (Table 2). In particular, ANOVA applied to VAS1, VAS2 and VAS3 showed a significant effect of the factor Test ($F(1,18)=9.8$, $p<0.01$, $F(1,18)=12.9$, $p<0.002$; $F(1,18)=16.6$, $p<0.001$ respectively) due to the lower level present in Test II than in Test I. ANOVA applied to Present Pain Intensity (PPI) values showed a significant effect of Test ($F(1,18)=20.4$, $p<0.001$) due to the decrease from Test I to Test II. The reduction was highly significant in both sexes ($p<0.003$ and $p<0.01$, respectively).

As reported in Table 2, ANOVA applied to QUIDs showed a significant effect of Sex ($F(1,18)=6.38$, $p<0.02$) and Test ($F(1,18)=5.17$, $p<0.03$) due to the higher levels in females than in males and the decrease from Test I to Test II in both sexes. The significance of the factor Test ($F(1,18)=8.64$, $p<0.008$) in QUIDm was due to the decrease from Test I to Test II. ANOVA applied to PRIr-T revealed a significant effect of Test ($F(1,18)=9.13$ $p<0.007$) due to the decrease from Test I to Test II; in this parameter females had non-significantly higher levels than males ($p=0.07$).

Figure 1 Common points used to treat pain patients by acupuncture: GB20, DU24, ST36, BL40.

Table 1 General information about the patients

S No.	Sex	Age (y)	Weight (kg)	Height (m)	BMI	Physical activity	Smoker	Pain location	Acupuncture points
1	M	64	73	1.75	23.8	No	No	Hand	DU24(1), HN3(1), LI3(2), SJ2(1), Ashi(1), ST36(2)
2	M	62	66	1.72	22.3	No	No	Trigeminal	DU24(1), ST7(1), ST6(1), ST4(1), LI20(1), ST34(1), SP10(1), ST36(1), SP9(1), GB34
3	M	66	81	1.78	25.6	Yes	No	Trigeminal	DU20, HN3, HN5(2), LI20, ST6, ST4, ST7, LI4(2), SP6(2), LR3(2)
4	M	52	80	1.77	25.5	No	No	Cervical	GB20(2), GV16, GB21(2), GV8, GV3, GV14, BL62(2), K16(2)
								Discal Hernia	
5	M	54	80	1.75	26.1	Yes	No	Neck pain	GB20(2), GB21(2), GV14(1), GV8, GV9, GV11, SI11, BL40(2), TE14, BL25(2), ST36(1)
6	M	62	88	1.81	26.6	Yes	No	Fasciitis	DU20, GB20(2), BL40(2), GB34(1), BL57(1), BL60(2), KI3(2)
7	M	42	90	1.76	29.1	No	No	Shoulder pain	GB20(2), GB21(2), GV8, GV16, GV3, GV14, GV20, BL62(2), K16(2), ASHI
								Cervical	
8	M	63	88	1.82	26.6	Yes	No	Sciatic nerve	DU20(1), DU3(1), BL25(2), BL54(1), GB30(1), BL40(2), BL57
9	F	61	57.5	1.57	23.3	No	No	Muscular pain	DU20(1), DU24(1), HN3(1), RN17(1), HT7(2), ST25(2), RN9(1), RN5(1), SP9(2), ST40(2)
								General	
								Anxiety	

Table Continued

S No.	Sex	Age (y)	Weight (kg)	Height (m)	BMI	Physical activity	Smoker	Pain location	Acupuncture points
10	F	47	65	1.72	22	Yes	No	Cervical discal hernia	GB20(2), GV14, GV3, BL25(2), BL49, UE8(2), BL31(2)
								Trigeminal	
11	F	55	58	1.62	22.1	Yes	No	Lumbago	GB20(2), SI11(2), GV17(1), GV8(1), GV7(1), BL25(2), BL40(2), BL54(2)
								Neck Pain	
12	F	62	65	1.7	22.5	Yes	No	Neck pain	GV20(2), GV14(1), BL25(2), GV3(1), BL23(2), PC6(1), SP10(2), GB34(1)
								Lumbago	
13	F	42	50	1.57	20.3	No	No	Lumbago	GB20(2), GV14(1), TF1(1), SC10(1), GV8(1), GV9(1), BL25(2), BL23(2), BL54(1), BL40(2), KI7(1), KI6(1), KI3(1)
14	F	53	52	1.63	19.6	No	Yes	Lumbago	BL23(2), DU3(1), BL25(2), BL30(1), GB30(1), BL40(2), BL57(1), GB34(1)
15	F	49	80	1.71	27.4	No	Yes	Lumbago	DU9(1), DU8(1), BL23(2), DU3(1), BL25(2), BL35(2), BL40(2), GB34(2)
16	F	57	42.5	1.57	17.2	No	No	Cervical	GB20(2), GV14, LI15(DX), LI16(DX), LI11(2), LI4(2), SI11(2), GV9, GV8, GV6, GV3, BL23(2), BL25(2), GB34(DX), VE(DX)
17	F	60	47	1.5	20.9	No	Yes	Shoulder pain	VE(2), LI15(2), LI11(2), ST40(2), LI16(2)
18	F	51	68	1.66	24.7	YES	Yes	Headache	GV24, CV4, ST28(2), PC6(2), SP10(2), SP6(2), HN5(2), ST40(2), HT7(2)
19	F	65	57	1.71	19.5	NO	Yes	Lumbago	BL23(2), DU3(1), BL25(2), BL30(1), GB30(1), BL40(2), BL57(1), GB34(1)
20	F	42	78	1.62	29.7	Yes	No	Hand pain	DU24(1), HN3(1), LI3(1), SJ2(1), ST36(1)
21	F	61	54	1.6	21.1	No	Yes	Lumbago	BL23(2), DU3(1), BL25(2), BL30(1), GB30(1), BL40(2), BL57(1), GB34(1)
								Leg Pain	
22	F	56	55	1.65	20.2	No	Yes	Shoulder pain	GB20(2), DU14, SJ15(2), SI11(2), DU9, DU8, PC5(2)

MIF: MIF values were higher in males than females, although ANOVA did not reach significance (Figure 2). In both sexes, the levels decreased significantly from Test I to Test II (ANOVA: Test (F(1,15)=13.6, p<0.01).

Table 2 VAS (I morning, II afternoon, III night), PPI (Present Pain Index), Total Pain Rating Index rank (PRIr-T**)** and QUID values. * p<0.05 and **p<0.005 Test I vs Test II, independently of Sex. Data are mean±SEM

Subjects	Test	VAS			PPI **	PRIr-T **	QUID S *	QUID A	QUID E	QUID M **
		I **	II **	III **						
MEN (n=8)	I	5.6±1.0	5.9±1.2	6.5±1.2	2.8±0.5	8.5±3.3	3.4±1.0	1.6±1.1	1.6±0.9	1.9±0.8
	II	2.5±0.7	2.1±0.6	1.7±0.4	1.4±0.3	3.8±0.9	2.3±0.6	0.6±0.6	0.4±0.3	0.5±0.3
WOMEN (n=14)	I	6.0±0.7	6.1±0.7	6.1±0.8	2.6±0.3	15.3±2.3	7.5±0.9	3.0±0.7	2.3±0.8	2.5±0.7
	II	4.7±0.7	4.4±0.8	4.0±1.0	1.9±0.2	9.9±2.5	4.9±1.2	1.7±0.62	2.3±0.8	1.1±0.5

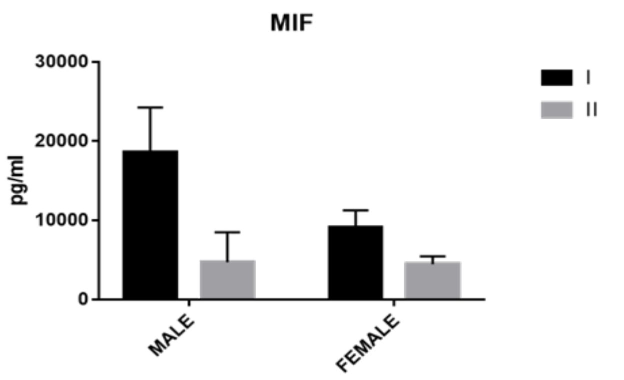

Figure 2 MIF salivary levels determined in male and female patients before the first (I) and tenth (II) acupuncture treatments. Data are mean±SEM.

Temperature, HR, BP

Vital parameters recorded in all patients were not modified by treatment (Table 3). Indeed, temperature determined in the left and right arm showed no sex differences and no variations between the first and the second test. HR showed a trend towards lower levels in males in the second test, while the contrary occurred in females. These differences did not reach significance. BP tended to decrease in males and increase in females, but no significant differences were found.

POMS: All POMS values were higher than 55, or lower in the V-A subscale, suggesting a non-physiological condition (Table 4). No differences were found except for ANOVA applied to the Depression-Dejection (D-D) subscale, which showed a significant interaction Sex x Test (F(1,18)=4.6, p=0.04) due to the increase in males (p<0.04) but not in females from Test I to Test II. For the other subscales, no significant differences were found.

SF-36: As reported in Table 5, ANOVA applied to SF-36 (BP, GH, VT, SF, ML subscales and PCS-36 and MCS-36 component summaries) showed a significant effect of Test (F(1,18=23.0, p=0.001; F(1,18)=8.5, p=0.009; F(1,18)=11.3, p<0.003; F(1,18)=15.5, p<0.001; F(1,18)=8.7, p<0.008; F(1,18)=12.3, p<0.003; F(1,18)=5.2, p<0.04 respectively) due to the increase from Test I to Test II. ANOVA applied to the PF subscale showed a significant effect of Sex (F(1,18)=4.8, p<0.04) due to the higher level in males than in females (p<0.02).

Table 3 Axillary temperature, heart rate and blood pressure. Data are mean±SEM

Subjects	Test	Axillary Temperature (°C)		Heart Rate (bpm)	Blood Pressure (mmHg)
		SX	DX		
Men	I	35.2±0.3	35.3±0.3	61.5±2.0	103.8±3.6
(n=8)	II	35.1±0.3	35.1±0.3	61.4±1.9	99.9±4.0
Women	I	35.7±0.1	35.6±0.2	66.6±3.9	96.6±2.8
(n=14)	II	35.3±0.2	35.4±0.2	67.8±3.4	105.4±5.6

Table 4 POMS values.

Subjects	Test	POMS T-A	POMS D-D	POMS A-H	POMS V-A	POMS F-I	POMS C-B
MEN (n=8)	I	65.7±1.1	70.6±0.9	83.1±1.2	41.9±2.4	69.1±2.5	63.5±1.2
	II	63.5±1.0	73.0±0.5 *	81.8±0.9	47.1±3.0	72.1±0.8	64.5±1.0
WOMEN (n=14)	I	63.1±0.8	71.6±0.8	82.6±1.0	47.9±1.9	72.5±1.2	64.5±1.5
	II	64.4±0.8	70.9±1.1	82.3±0.8	49.2±1.6	69.9±1.1	64.8±1.2

Tension-Anxiety (T-A), Depression-Dejection (D-D), Anger-Hostility (A-H), Vigor-Activity (V-A), Fatigue-Inertia (F-I), Confusion-Bewilderment (C-B). In each subscale, values higher (T-A, D-D, A-H, F-I, C-B) or lower (V-A) than 55 were considered significantly altered with respect to the normal population. *p<0.05, Test I vs Test II. Data are mean±SEM.

Table 5 SF36 values.

	Men (n=8)		Women(n=14)	
	I	II	I	II
SF36 PF #	78.1±11.4	94.4±1.7	66.8±7.0	66.3±7.1
SF36 RP	68.8±11.3	84.4±12.4	48.2±9.2	56.3±12.7
SF36 BP **	28.9±6.2	64.5±7.6	34.4±6.1	54.3±7.7
SF36 GH *	57.3±5.2	67.6±5.2	55.1±4.9	61.7±5.5
SF36 VT *	53.1±5.3	61.3±5.9	42.1±5.5	54.6±5.4
SF36 SF **	49.6±7.1	79.6±7.4	57.9±5.3	69.6±7.4
SF36 RE	66.4±12.6	79.0±12.5	71.2±9.2	77.7±10.3
SF36 MH *	61.6±6.0	66.0±6.3	59.7±4.3	72.7±4.0
PCS-36 *	41.1±3.1	50.7±1.6	37.1±2.7	39.7±3.0
MCS-36 *	42.0±2.5	45.8±3.8	43.5±2.6	49.6±2.2

Physical Component Summary (PCS-36), includes physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH); Mental Component Summary (MCS-36), refers to vitality (VT), social functioning (SF), role emotional (RE), mental health (MH). For each domain, including bodily pain (BP), and the component summaries, a higher score indicates a better quality of life and lower limitations. *p<0.05 and **p<0.001 significantly increased from Test I to Test II, independently of Sex. # p<0.05, higher level in men vs women, independently of Test. Data are mean±SEM.

Discussion

The main result of the present study is the positive effect of acupuncture on pain and MIF levels, as well as quality of life indicators, in both men and women. Pain is one of the main reasons prompting men and women to seek acupuncture treatment. The pathophysiological mechanisms underlying this analgesic effect are not clear. We have recently shown[21] that age and sex are important factors modulating the acupuncture-induced effects and that these effects can be related to steroid hormone changes. In the present study, male and female subjects were evaluated before and at the end of a normal cycle of acupuncture treatments (10 sessions, 2-3 times per week) to study the changes in the levels of pain and macrophage inhibitory factor (MIF). Other physiological measures (i.e. temperature, heart rate, blood pressure) were carried out to evaluate the general status of the patients. Questionnaires were administered to assess the mood state and quality of life of the patients before and after the therapy.

Acupuncture treatment was effective in all patients, as indicated by the decreased pain intensity in both sexes at the end of the treatment. Indeed, the results of the different questionnaires (VAS, QUID) carried out before and after treatment all agreed in showing that the patients felt less pain independently of their pathology. Moreover, the questionnaire on the quality of life (SF-36) also revealed much improvement from the first to the second test, supporting a beneficial effect of the acupuncture treatment on all subjects.

MIF salivary levels showed a drastic decrease in both sexes. MIF is a pleiotropic inflammatory cytokine with regulatory roles in innate and adaptive immune responses.[22] We previously reported higher MIF levels in men than in women and a positive correlation with testosterone levels.[23] In the present study, we confirm the marked sex difference, with higher levels in males than in females. Moreover, the significant decrease from the first (beginning of treatment) to the second (end of treatment) test in both sexes strongly indicates an active role of this cytokine in pain. Indeed, MIF production is greatly increased in inflammatory and autoimmune diseases, and the high MIF levels appear to play a critical role in the pathogenesis of the disease. MIF is also an essential determinant for neuropathic and inflammatory pain, as shown in interstitial cystitis/bladder pain syndrome[17] and in stress-induced neuropathic pain.[24]

Given the harmful effects of excessive MIF levels, many efforts have been made to develop appropriate therapeutic strategies to diminish MIF production and/or activity for the improvement of health conditions in inflammatory diseases and the persistent pain often associated with this type of disease. Multiple studies in animals show that pharmacological inhibition of MIF with anti-MIF antibodies or inhibition of MIF activity by small molecule inhibitors have proven effective in various diseases such as rheumatoid arthritis and systemic lupus erythematosus.[25] Genetic deletion of MIF in mice also led to an improvement in the inflammatory response and progression of acute pancreatitis[14] and blockage of the pain responses in interstitial cystitis/bladder pain syndrome.[17]

Therefore, any strategy aimed at countering the effects of excessive levels of MIF is extremely important for the attenuation of symptoms in inflammatory diseases and painful conditions often associated with them. Acupuncture appears to have an important effect on this cytokine, supporting the use of this non-pharmacological therapy to treat pain.

Conclusion

This study demonstrates the ability of acupuncture treatment to decrease pain. The strong decrease in the cytokine MIF suggests an anti-inflammatory action in both sexes as the result of treatment.

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Conflicts of interest

All authors declare no conflicts of interest.

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