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Waggle needling wields preferable neuroprotective and anti-spastic effects on post-stroke spasticity rats by attenuating γ-aminobutyric acid transaminase and enhancing γ-aminobutyric acid

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Waggle needling, a classical anti-spastic needling technique characterized by combination of acupuncture with joint movement, has gained increasing popularity of spasticity treatment in China. This study was designed to compare the anti-spastic effect of waggle needling to the routine needling and to explore its underlying mechanism. We established post-stroke spasticity model based on ischemia stroke operation (middle cerebral artery occlusion). Rats were divided into six groups: normal control group, sham-operated control group, ischemia stroke model group, waggle needling group, routine needling group and baclofen group. Neurological function and muscle tone were assessed by the Zea Longa score and modified Ashworth scale, respectively. Indirect muscle tone was testified with electrophysiological recording. Cerebral infarction was measured by 2,3,5-triphenyltetrazolium chloride staining. The concentrations and expressions of γ-aminobutyric acid transaminase (GABAT) and γ-aminobutyric acid (GABA) were detected by enzyme-linked immunosorbent assay and western blot assay. Waggle needling markedly alleviated neurological deficits, decreased cerebral infarction and eased muscle tone; simultaneously, attenuated GABAT and enhanced GABA expression in the cortical infarct regions in comparison with the routine needling ($P < 0.01$), yet showed similar therapeutic effect to the baclofen group ($P > 0.05$). These results preliminary supported that waggle needling as a potential promising non-pharmacological intervention for the treatment of cerebral ischemia and spasticity. *NeuroReport* 31: 708–716

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Introduction

Spasticity is a common complication following stroke [1], and one of the consequences of the upper motor neuron (UMN) syndrome. Following an UMN lesion, a loss of inhibition control after stroke, particularly the reticulospinal tract hyperexcitability, is likely to be the primary mechanism, while altered intraspinal network processing is secondary factor that contributes to post-stroke spasticity [2]. These changes can lead to α-motor neurons hyperexcitability and thus excessive muscle tone and contraction [2]. Spasticity usually hinders rehabilitation process in patients after stroke [3] with higher health care cost [4]. Some orally active agents such as baclofen is commonly used for the management of spasticity, however, drug-induced adverse effects like muscle weakness and hepatotoxicity remains problematic [5]. Therefore, a more effective and safe therapy is desired by both doctors and patients.

γ-aminobutyric acid (GABA) is one of the chief inhibitory neurotransmitters in the central nerve system (CNS), plays pre- or postsynaptic inhibitory effects [6]. It has been shown that decreased GABA neurotransmission was involved in the etiology of several neurological diseases such as spasticity, epilepsy and others [7]. Enhanced expression of GABA promotes spasticity suppression in patients with post-stroke spasticity [8]. GABA transaminase (GABAT) is the key catabolic enzyme of GABA, inhibition of GABAT is known to boost GABA concentration in the brain [9], and thereby alleviates spasticity. Therefore, attenuating GABAT activity and enhancing GABA expression are a promising way to relieve spasticity following stroke.

As a traditional Chinese medical therapy, acupuncture has a long history and remains widely used in contemporary

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clinical practice. It has been commonly applied for treating post-stroke spasticity in China. Previous clinical and experimental studies provided clear evidence that acupuncture could ameliorate post-stroke spasticity [8,10]. Clinically, the stimulating methods and specific needling techniques applied on the acupoints are crucial for acupuncture efficacy. Catgut implantation at acupoints showed satisfied anti-spastic effect via up-regulating GABA or its receptor [11]. Waggle needling is a classical anti-spastic needling technique recorded in *Huangdi NeiJing*, emphasizing the effective combination of acupuncture with joint movement, especially good for treating muscle and sinew disorders. In light of clinical experience [12], anti-spastic effects exerted by waggle needling was comparatively better than routine needling, particularly in increasing joint range of motion (ROM) and maintaining limb balance. Nonetheless, its underlying mechanism still remains unclear and worthy of further study.

Given that there are data suggesting the modulation of GABA and its metabolism, we hypothesized that acupuncture, particularly waggle needling would attenuate GABAT and enhance GABA, alleviating post-stroke spasticity.

Materials and methods

Animals and reagents

Specific-pathogen-free adult male Sprague–Dawley rats (n = 120) weighing 270–290 g were purchased from Beijing Vital River Laboratory Animal Technology. Both the animal care and study protocol were approved and executed strictly in conformity with the Animal Ethics Committee of Beijing University of Chinese Medicine (Approval No. BUCM-4-2019062701-2048), and all efforts were made to minimize animal suffering. Under constant temperature (23 ± 2°C) and constant humidity (40–60%), rats were housed on a standard 12-h light-dark cycle (dark cycle 8:00 p.m.–8:00 a.m.), with access to food and water *ad libitum*. Baclofen (Weidar Chemical & Pharmaceutical Co., Ltd., Taichung, Taiwan, China) was diluted using normal saline to a final concentration of 1 mg/ml.

Ischemia stroke model

With certain minor modifications, ischemia stroke model was established based on the middle cerebral artery occlusion (MCAO) as described previously [13]. Briefly, rats were anesthetized with isoflurane (1–2%) in O_2, the right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were isolated. With the help of 5 ml syringe needle, inserted the nylon monofilament (0.36 ± 0.02 mm in diameter, 45 mm in length; Beijing Shandong Biological Technology Co., Ltd., Beijing, China) into the CCA. Pull out the needle and advanced the nylon monofilament through the ICA to the origin of middle cerebral artery, and stopped until the blunt end of the monofilament met mild resistance, almost 18–20 mm from the carotid bifurcation. The incisions were sutured in layers. Benzylpenicillin Sodium (8000 U each rat) was injected intraperitoneally for 3 days with the purpose of anti-infection. Electric blanket was utilized until the rats awaken after surgery. Functional assessments with the Zea Longa neurological deficit score and modified Ashworth scale (MAS) were performed by the same investigator. Rats with Zea Longa scores of 1–3 and MAS scores of 1–4 were included.

Behavioral assays

Neurological function assessment was performed in each rat at the day before MCAO, day 3 (after surgery but before treatment) and day 9 after MCAO (7 days after treatment) with the Zea Longa score [14] as follows: 0, no neurological deficit; 1, failure to extend left forepaw fully; 2, circling to the left; 3, falling to the left; 4, loss of walking and consciousness.

Muscle tone was evaluated with the MAS [15] at the same three-time points as scoring of neurological deficits, namely, the day before MCAO, day 3 and day 9 after MCAO, separately. Scale is graded from 0 to 4 and presented as: 0, no increase in muscle tone, with limb moving freely; 1, slight increase in muscle tone, with a catch and release or minimal resistance at the end of the ROM during flexion and extension; 1+, slight increase in muscle tone with a catch and minimal resistance throughout the remainder (less than half) of the ROM; 2, more marked increase in muscle tone through most of the ROM, with affected limb moving easily; 3, considerable increase in muscle tone, with difficulty in passive movement; 4, limited joint movement. Maximum score in the study was 5, and the 1+ was converted to 2 [16].

Experiment groups

The rats were randomly divided into following six groups: normal control (normal, n = 14), sham-operated control (sham, n = 14), ischemia stroke model (model, n = 23), waggle needling (n = 23), routine needling (n = 23) and baclofen (n = 23) groups.

Rats in the normal group received no surgery. Rats in the sham group underwent neck dissection and the exposure of CCA, ECA and ICA, without MCAO. Rats in the model group and three treatment groups underwent MCAO.

Three days after MCAO, the rats in two acupuncture groups received waggle needling or routine needling at *Yanglingquan* acupoint (GB 34) respectively for 7 days, 30 minutes per session, once daily; while the rats in the baclofen group underwent baclofen gavage and then immobilization up to 30 minutes, once a day for a period of 7 days.

Meanwhile, the rats in the normal, sham and model groups received no therapeutic interventions, but underwent equal time (30 minutes) for immobilization

with rats in the waggle needling, routine needling and baclofen groups.

Treatments

Waggle needling manipulation

(1) Needle (ϕ 0.25 × 13 mm; Zhongyan Taihe, Beijing, China) was inserted into GB 34 perpendicularly, for a depth of 4–5 mm beside the capitulum fibula of the left hindlimb. (2) Repeatedly change the direction of needling from left to right around GB 34 by lifting-thrusting method for 30 seconds. (3) After that, the needle was lifted just beneath the skin surface, followed by full and slow passive flexion and extension of the ankle joint, lasting for 30 seconds, then the needle was retained at the depth of 4–5 mm for 30 minutes (Fig. 1).

Routine needling manipulation

Needle was inserted into GB 34 perpendicularly for a depth of 4–5 mm and then manipulated with vertical lifting-thrusting methods for 30 seconds totally. Then, the needle was retained for 30 minutes.

Baclofen gavage

Baclofen group is designed as a positive control in our study. Rats in this group received baclofen (10 mg/kg body weight) by intragastric administration.

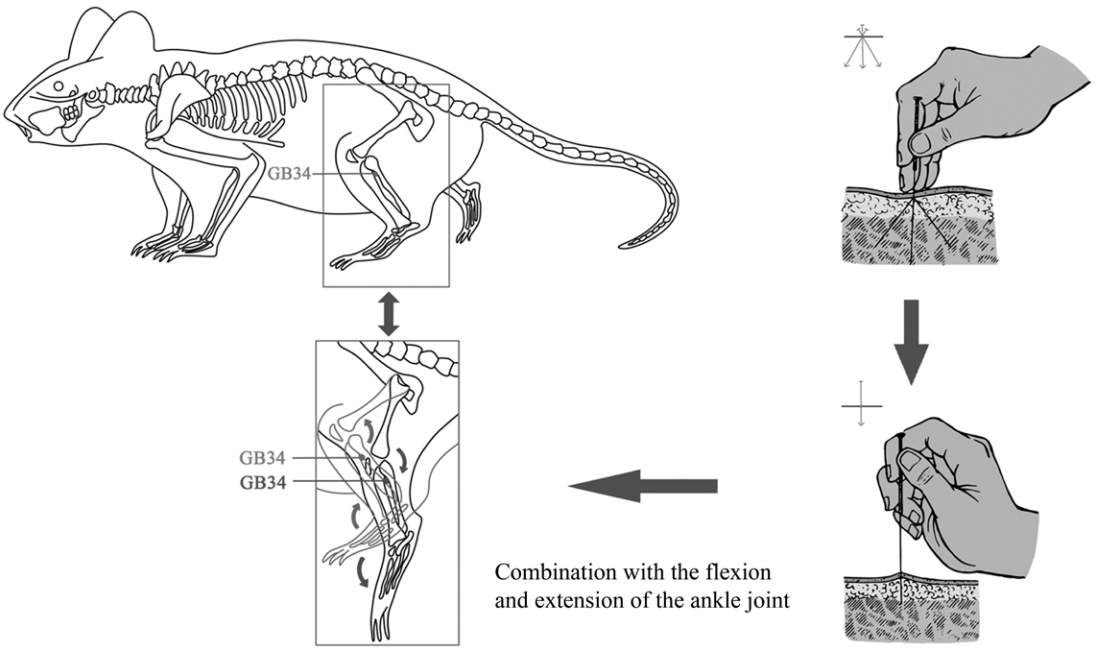
2,3,5-triphenyltetrazolium chloride staining for cerebral infarction

After final behavioral assays, six rats per group were deeply anesthetized with 1% pentobarbital sodium (40 mg/kg) and sacrificed for 2,3,5-triphenyltetrazolium chloride (TTC) (AMRESCO, Solon, Ohio, USA) staining. The brain was quickly harvested and froze for 20 minutes. Six serial slices of 2 mm thickness were obtained and incubated in 0.3% TTC solution at 37°C in the dark environment. Then fixed the slices in the 4% paraformaldehyde solution and photographed. ImageJ software (1.52q; National Institutes of Health, Bethesda, Maryland, USA) was used for the measurement of percent infarct volumes.

Indirect muscle tone assay

Indirect muscle tone assay was administered by electrophysiological recorder before and after treatment (day 3 and day 9 after modeling) as we previously described [17]. Briefly, after anesthesia, one stimulating needle electrode was vertically inserted into the quadriceps femoris of the rat left hindlimb, the other was transversely inserted into the tail. A low-compliance cotton thread was attached to the lower end of the left hindlimb, and the cotton thread was connected to the electrophysiological recorder system (BL-420S; Taimeng Technology Corporation of

Fig. 1



Point location of GB 34 and manipulation of waggle needling technique.

Chengdu, Chengdu, China) through a tension sensor. After 0.5 g of afterload was settled, the quadriceps femoris was stimulated regularly (3 mA, 30 seconds) and then the electrical signals induced by the affected quadriceps femoris were recorded, which indirectly reflected the changes of muscle tone in rats. Relatively stable wave with 10 seconds was selected and peak-to-peak values were calculated automatically.

Enzyme-linked immunosorbent assay for γ-amino butyric acid transaminase and γ-amino butyric acid

After anesthesia, the right cortical infarct regions of rats were obtained. According to the manufacturer's instructions, Rat GABAT enzyme-linked immunosorbent assay (ELISA) Kit (JL47786; Jianglai Biological Co., Ltd, Shanghai, China) and Rat GABA ELISA Kit (JL12343) were used for the measurement of GABAT and GABA protein concentrations.

Western blot assay for γ-aminobutyric acid transaminase and γ-aminobutyric acid

Certain amounts of the right cortical infarct regions and radioimmunoprecipitation assay lysates were centrifuged at 12 000 rpm for 15 minutes at 4°C and quantified by bicinchoninic acid assay (PC0020; Beijing Solarbio Technology Co., Ltd, Beijing, China). After electrophoresis, 30 μg of protein were transferred to a polyvinylidene difluoride membrane. Then the membrane was blocked for about 2 h at room temperature with 3% BSA in TBST solution and incubated with rabbit monoclonal antibody anti GABAT (1:1000; Abcam, Cambridge, UK) or rabbit polyclonal antibody anti-GABA (1:1000; Abcam), with GAPDH (TA-08; Beijing ZSGB Biotechnology Co., Ltd, Beijing, China) as internal control, at 4°C overnight. The membranes were washed with TBST solution three times every 7 minutes and then incubated with the goat anti-rat secondary antibody (1:5000; Beijing ZSGB Biotechnology Co., Ltd) for 1 h, at 37°C. Enhanced chemiluminescence kit (5200; Tanon Science & Technology Co., Ltd, Shanghai, China) was applied for visualization of protein bands, which were captured and analyzed by image analysis system (1.52p; Softonic, Barcelona, Span). The optical density value of the protein bands were normalized to GAPDH and calculated.

Statistical analysis

Statistical analysis was conducted using SPSS 20.0 software (IBM, Armonk, New York, USA). Significance was determined with Chi-square test or Wilcoxon signed-rank test or one-way analysis of variance. The least significant difference-t post hoc test was used for pairwise comparisons. Data was presented as mean ± SD. *P*-value less than 0.05 was considered statistically significant differences.

Results

Quantitative analysis of experimental rats

No rats died in the normal and sham groups. Six rats died and three rats exhibited no spasticity in the model group; four rats died and five rats exhibited no spasticity in the waggle needling group; five rats died and four rats exhibited no spasticity in the routine needling group; five rats died and six rats exhibited no spasticity in the baclofen group. There was no statistical difference of the survival rates among the up-mentioned groups ($\chi^2 = 0.511$, $P > 0.05$). All included rats met the criteria: Zea Longa scores (1–3), MAS scores (1–4) and statistical differences were no found among each group referring to the Zea Longa ($P > 0.05$) and MAS ($P > 0.05$). Therefore, the final analyses included 14 rats among each group except for the baclofen group (n = 12). Six rats each in all groups were used for TTC staining. Six rats each in all groups were used for electrophysiological recordings. Six rats each in all groups were sacrificed for ELISA and western blot assay.

Waggle needling decreased cerebral infarction and alleviated neurological deficits

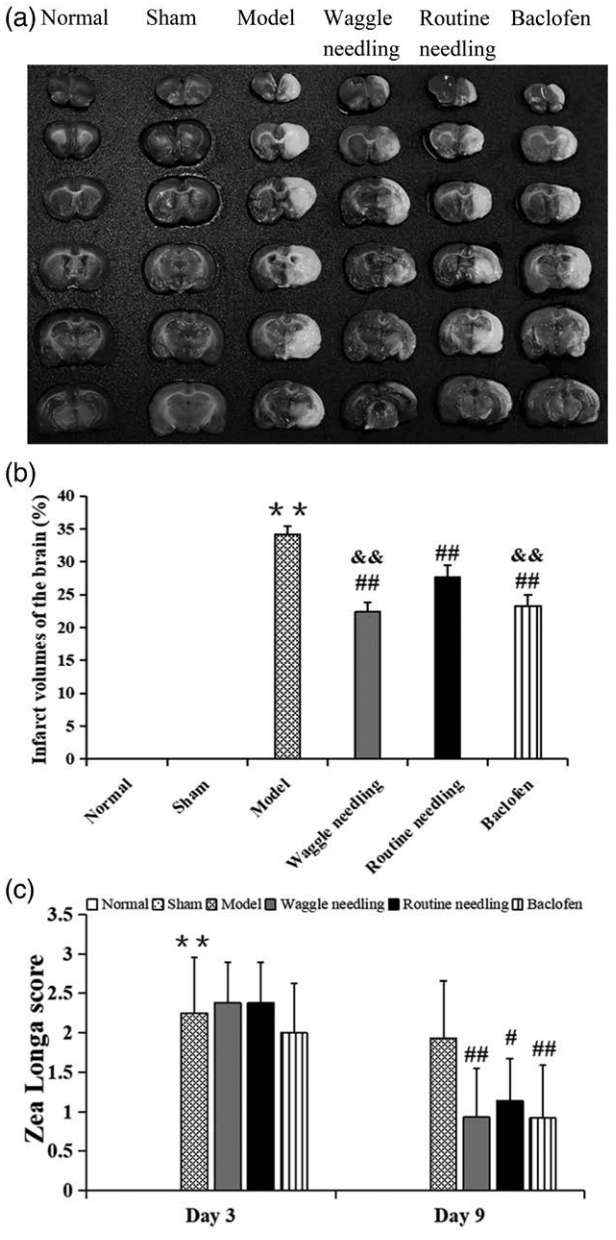
Rats in the normal group and sham group showed no cerebral infarction, whereas, all of the rats in model, waggle needling, routine needling and baclofen groups manifested obvious cerebral infarction and atrophy as presented in Fig. 2a. The TTC results showed that the infarct volumes varied significantly across different groups ($F[3,20] = 71.64$, $P < 0.05$). Following 7 days' treatment, the cerebral infarction volumes were significantly decreased in all three treatment groups compared with model group ($t = 13.14$, $t = 7.25$ and $t = 12.07$, respectively; all $P < 0.01$; Fig. 2b). Furthermore, both waggle needling and baclofen showed better effects on reduction of cerebral infarction than routine needling ($t = 5.88$, $t = 4.82$; all $P < 0.01$; Fig. 2b). No significant difference was found between waggle needling group and baclofen group ($t = 1.06$, $P > 0.05$).

Zea Longa score was 0 in all rats at the day before MCAO, thus it was not presented in Fig. 2c. Three days after surgery and before treatment, Zea Longa score was still 0 in the normal and sham groups. At the same time point, Zea Longa scores in the model, waggle needling, routine needling and baclofen groups were not significantly different ($P > 0.05$), while they were all significantly higher compared with the sham group ($P < 0.01$). After 7 days' treatment, the neurological deficits significantly relieved in waggle needling ($P < 0.01$), routine needling ($P < 0.05$) and baclofen group ($P < 0.01$; Fig. 2c), compared with the model group respectively. Meanwhile, waggle needling showed similar effect of improving the neurological function as baclofen ($P > 0.05$). These data indicated that waggle needling exerted therapeutic effect against cerebral ischemia injury, and this effect was more satisfied compared with routine needling.

Waggle needling eased muscle tone

MAS score was 0 in all rats at the day before MCAO, indicating muscle tone was normal. Three days after

Fig. 2



The statistic results of cerebral infarction and Zea Longa behavioral assay in each group. (a) At the end of experiments, cerebral tissues were stained by TTC. Images are representative of three dependent experiments. (b) Infarct volumes (n = 6 per group) were quantified using the ImageJ software (1.52q), which were represented as percentage of the total brain volumes. Data were analyzed with one-way analysis of variance test and expressed as mean ± SD. $^{**}P < 0.01$, compared with the sham group; $^{\#\#}P < 0.01$, compared with the model group; $^{\&\&}P < 0.01$, compared with the routine needling group. (c) Neurological deficits were assayed by Zea Longa score at day 3 and day 9 in each group [baclofen group (n = 12), the other five groups (n = 14)] during the experiment. Data were analyzed with Wilcoxon signed-rank test and expressed as mean ± SD. $^{**}P < 0.01$, compared with the sham group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the model group. TTC, 2,3,5-triphenyltetrazolium chloride.

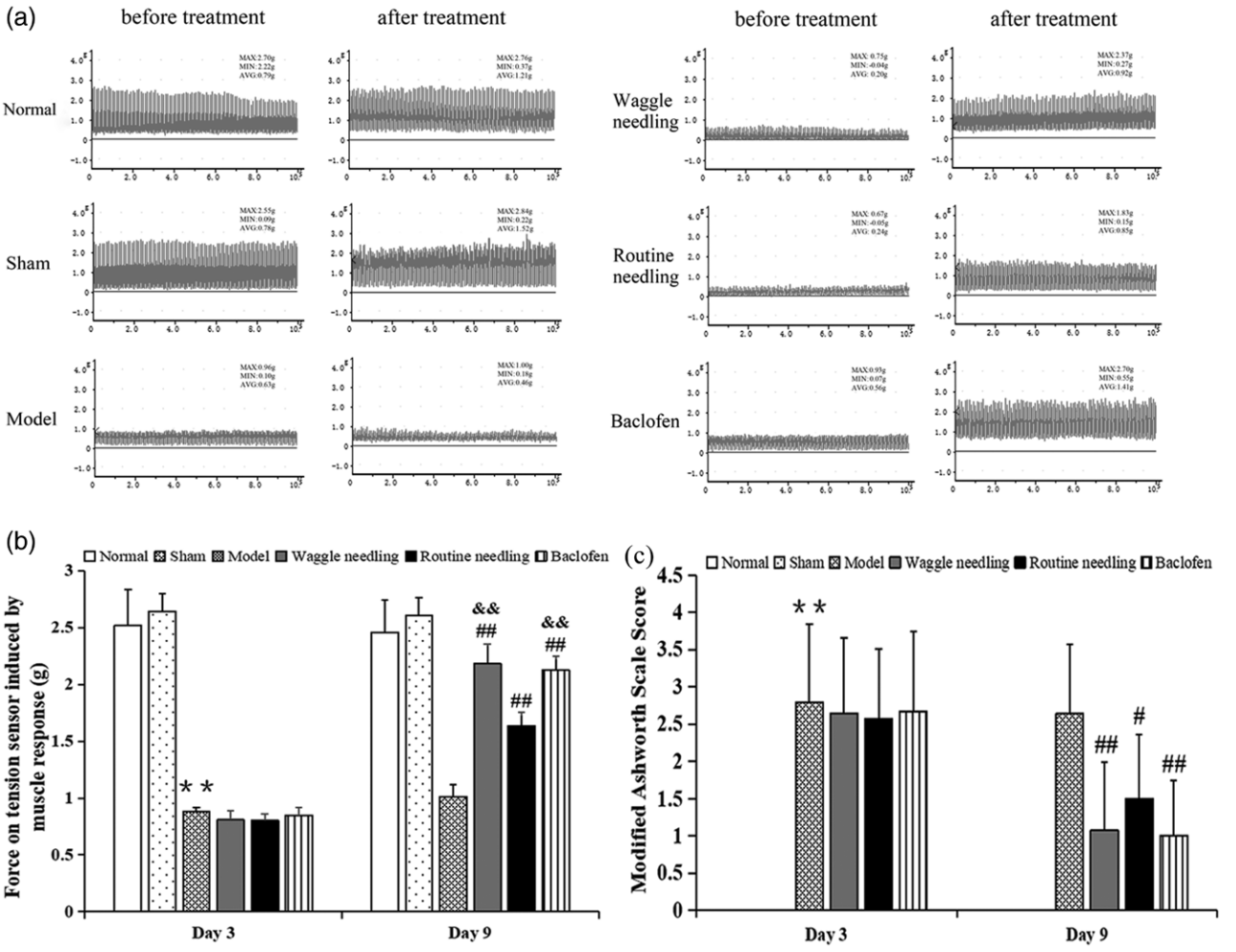
operation and before treatment, MAS score was still 0 in the normal and sham groups. At the same time point, MAS scores in the model group and three treatment groups existed no statistical differences ($P > 0.05$), but they were all obviously higher compared with the sham group ($P < 0.01$; Fig. 3c). Following 7 days' treatments, muscle tone was markedly decreased in the waggle needling group ($P < 0.01$), routine needling group ($P < 0.05$) and baclofen group ($P < 0.01$; Fig. 3c), compared to the model group, respectively.

For better confirming the above assay, electrophysiological recorder was performed to measure the indirect muscle tone as shown in Fig. 3a, with the underlying mechanism that the higher of the muscle tone is, the smaller of the muscle reaction to the electric stimulation, and vice versa. The results of electrophysiological recordings varied significantly across the different groups before and after treatment ($F[5,13] = 152.49$, $F[5,30] = 70.50$, respectively; $P < 0.05$). Rats in the model group presented a significant reduction in the amplitude of muscle response waves to the same electric stimulation than those in the sham group ($t = 26.54$, $P < 0.01$) at day 3 after surgery, for example, force on tension sensor was 0.88 ± 0.04 in the model group, while 2.64 ± 0.16 in the sham group, indicating an increase of muscle tone and occurrence of spasticity. After treatment, compared with the model group, muscle tone was significantly reduced in waggle needling group ($t = 11.83$, $P < 0.01$), routine needling group ($t = 6.31$, $P < 0.01$) and baclofen group ($t = 11.25$, $P < 0.01$; Fig. 3b). Compared with routine needling group, significant spasticity suppression was found in waggle needling group ($t = 5.52$, $P < 0.01$) and baclofen group ($t = 4.94$, $P < 0.01$; Fig. 3b). There was no statistical difference in muscle tone between waggle needling group and baclofen group ($t = 0.57$, $P > 0.05$). Overall, these data supported that waggle needling possessed a better suppression on muscle tone in post-stroke spasticity rats compared with routine needling.

Waggle needling attenuated γ-aminobutyric acid transaminase expression in the injured brain of rats with post-stroke spasticity

There were significant differences in the concentrations and expressions of GABAT among different groups in both ELISA and western blot assay ($F[5,30] = 9.73$ and $F[5,30] = 25.26$, respectively; $P < 0.05$). In comparison with the sham group, the concentration and expression of GABAT were significantly increased in the model group ($t = 5.44$ in ELISA and $t = 8.38$ in western blot assay, respectively; all $P < 0.01$; Fig. 4a, c and e). However, after treatment, compared with the model group, the concentration and expression of GABAT were significantly attenuated in waggle needling group

Fig. 3



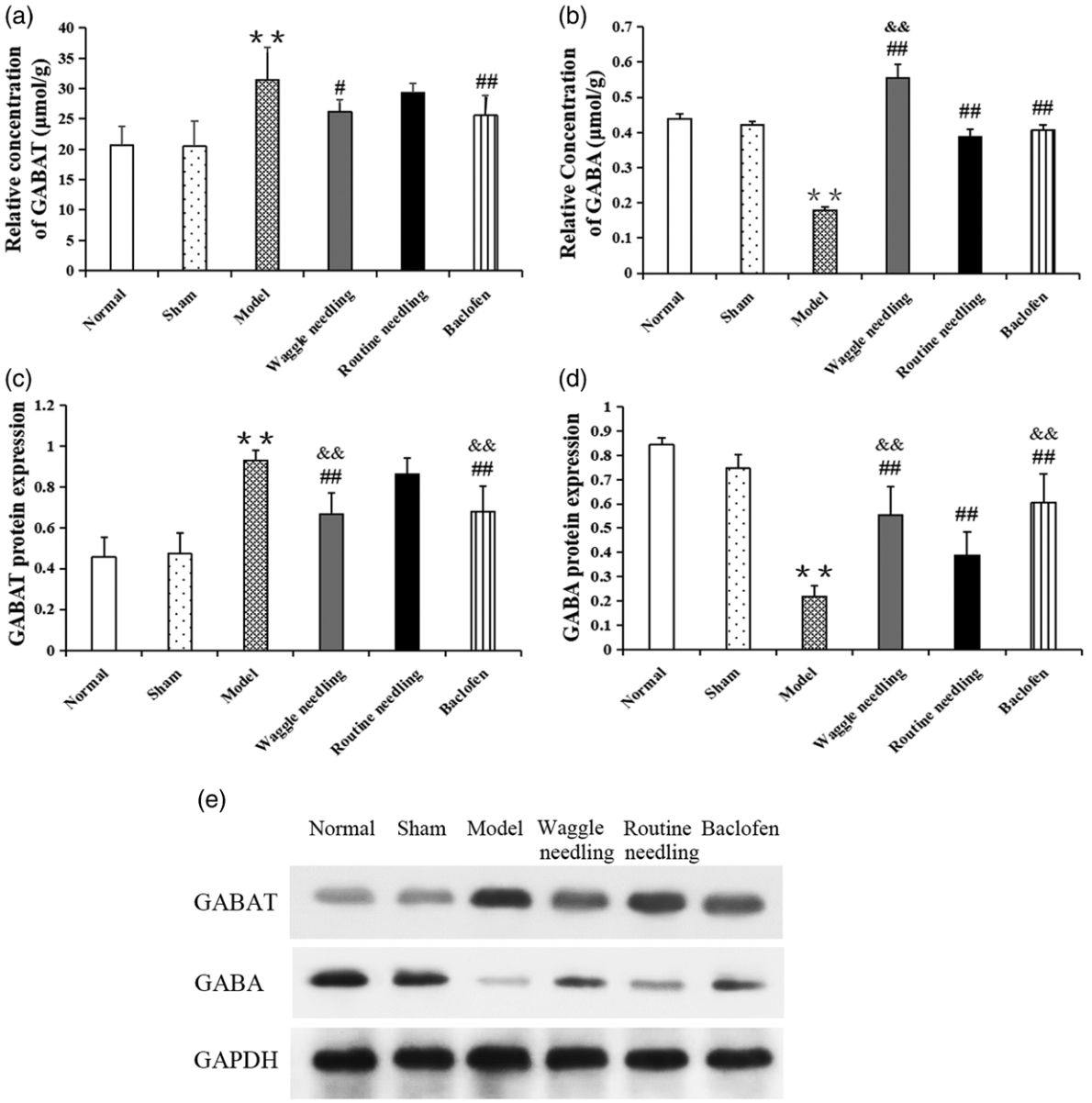
The statistic results of indirect muscle tone and MAS behavioral assay. (a) Under the same stimulation, the amplitude changes in muscle response waves between each group before and after treatment were shown. (b) The magnitude of force on tension sensor induced by muscle response in each group (n = 6), data were analyzed with one-way analysis of variance test and expressed as mean ± SD of three dependent experiments. (c) Muscle tension was assayed by MAS at day 3 before treatment and day 9 after treatment [baclofen group (n = 12), the other 5 groups (n = 14)], data were analyzed with Wilcoxon signed-rank test and expressed as mean ± SD. $^{**}P < 0.01$, compared with the sham group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the model group; $^{\&\&}P < 0.01$, compared with the routine needling group. MAS, modified Ashworth scale.

(t = 2.61, $P < 0.05$ in ELISA; t = 4.80, $P < 0.01$ in western blot assay) and baclofen group (t = 2.91 and t = 4.56 in ELISA and western blot assay, respectively; all $P < 0.01$; Fig. 4a, c and e). No significant difference was found in GABAT expression between routine needling and model group (t = 1.07, $P > 0.05$). Western blot assay also indicated that the expression of GABAT was significantly decreased in both the waggle needling group and baclofen group (t = 3.52 and t = 3.29, respectively; all $P < 0.01$; Fig. 4c and e) compared with the routine needling group, respectively. There were no statistical differences between the waggle needling group and baclofen group (t = 0.23, $P > 0.05$). These data showed that waggle needling could obviously attenuate GABAT expression.

Waggle needling enhanced γ-aminobutyric acid expression in the injured brain of rats with post-stroke spasticity

The expressions of GABA in ELISA and western blot assay showed significant differences among different groups (F[5,13] = 203.78 and F[5,30] = 44.34, respectively; $P < 0.05$). Both the ELISA and western blot assay results indicated that GABA concentration and expression in the model group were significantly decreased compared with the sham group (t = 38.90 and t = 10.85, respectively; all $P < 0.01$; Fig. 4b, d and e). For example, the concentration of GABA was 0.42 ± 0.01 and 0.75 ± 0.06 in the sham group, while 0.18 ± 0.01 and 0.22 ± 0.05 in the model group, corresponding to the ELISA and western blot assay, respectively. Whereas, following

Fig. 4



Protein concentrations and expressions of GABAT and GABA in each group. (a) and (b) were the bar graph of GABAT and GABA detected by ELISA. (c) and (d) were the bar graph of GABAT and GABA detected by western blot, which were corresponding to (e) (representative western blots for GABAT and GABA protein expression relatively to GAPDH protein). Data were analyzed with one-way analysis of variance test and expressed as mean ± SD of three dependent experiments (n = 6). $^{**}P < 0.01$, compared with the sham group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the model group; $^{\&\&}P < 0.01$, compared with the routine needling group. ELISA, enzyme-linked immunosorbent assay; GABAT, γ-aminobutyric acid transaminase; GABA, γ-aminobutyric acid

7 days' treatments, the concentration and expression of GABA were significantly enhanced in the waggle needling group, routine needling group and baclofen group (t = 22.66, t = 6.92; t = 21.00, t = 3.53; t = 30.71, t = 7.96 corresponding to ELISA and western blot assay, respectively; all $P < 0.01$; Fig. 4b, d and e) compared with those in the model group. Meanwhile, compared with the routine needling group, GABA expression was obviously increased in both the waggle needling group (t = 9.11 in ELISA, t = 3.39 in western blot assay; all $P < 0.01$) and baclofen group (t = 4.43 in western blot assay, $P < 0.01$; Fig. 4b, d and e). These data showed that waggle needling had better effect in enhancing GABA expression compared with routine needling.

Discussion

In this study, we found that waggle needling wields neuroprotective effects by alleviating neurological deficits and decreasing cerebral infarction. Furthermore, waggle needling displays anti-spastic effects by improving MAS and indirect muscle tone in a post-stroke spasticity model. The therapeutic effects may be related to GABAergic function, as presented by attenuated GABAT expression and enhanced GABA expression induced by waggle needling. To our best knowledge, this is the first animal study to compare the classical anti-spastic needling technique (waggle needling) with the routine needling technique and to explore its underlying mechanism.

Post-stroke spasticity induced abnormal postures and movement patterns hinder the rehabilitation process of patients [3], indicating the earlier the proper intervention apply, the better the curative effects will easily obtain. As a convenient and economic therapy, acupuncture could effectively counteract spasticity following stroke [8]. But it is worth noting that the effects of acupuncture could be particularly influenced by needling technique, such as depth of insertion, stimulation amount as well as manipulations [18]. According to the classical theory of acupuncture, proper application of specific needling technique for disease is essential to the efficacy of acupoints. In comparison with minimal needle-stimulation, acupuncture with strong needle-stimulation modulated functional connectivity in motor areas, increased blood flow of subcortical structures, therefore facilitated stroke survivors recovery from motor deficit [19]. A systematic review showed that either acupuncture or isolated passive limb movement in physiotherapy for treating spasticity achieved inferior curative effects [20], suggesting combination of several treatment methods may achieve more satisfied effects. Researchers found the disorder of the UMN would lead to peripheral muscle changes, for example, muscle fiber stiffness and shortening [21]. The complications following UMN lesions would be prevented by prolonged slow passive stretching, which is beneficial to facilitate positive movement of agonist or antagonist muscle groups so as to prevent muscle contracture and lessen spasticity-induced pain [22]. Interestingly, these researches provided certain pathological and physiological basis for waggle needling: the effective combination of acupuncture with joint movement and relatively strong stimulation, largely magnify the advantages of these two treatment methods. Previous studies confirmed the progressive increase in peripheral muscle resistance and neurological deficits in MCAO rats [11,13], we therefore performed the treatments from day 3 to day 9 after surgery. In acupuncture clinic, GB 34 was one of the most frequently used points for spasticity [23], thus, GB 34 was chose as the treatment acupoint in our study. The anti-spastic effect of waggle needling were confirmed and well accepted in the clinics [12], our results also suggest that the therapeutic effect on post-stroke spasticity of waggle needling was superior over the routine needling at the same acupoint. So the effectiveness of acupuncture might be mainly attributed to the needling techniques applied on the acupoints.

Although the imbalance between the central inhibitory and facilitatory system leading to hyperexcitability of the stretch reflex was considered to play a key role in post-stroke spasticity, accumulated recent experimental evidence has supported that decreased supraspinal inhibition and resultant α-motor neuron hyperexcitability after cerebral ischemia is shown as the primary mechanism [1]. A reduction in GABA in cerebrospinal fluid was found in patients with post-stroke spasticity [8]. Endogenous GABA release played neuroprotective role and was essential to prevent further neurodegeneration in rats after stroke [24]. Furthermore, electro-acupuncture could protect the ischemic cortex and hippocampus from further damaging through up-regulating GABA [25]. Thus, GABA may act as an important index of neuroprotective and anti-spastic effects. GABA is synthesized by glutamate decarboxylase, and metabolized by GABAT mainly existing in the mitochondrial matrix and widely spreading in the CNS; through a successive metabolism, GABA is converted to either succinic acid by GABAT and succinic semialdehyde dehydrogenase (SSADH), participating in tricarboxylic acid cycle and energy metabolism or the γ-Hydroxybutyrate [26]. It has been reported that valproic acid-induced inhibition to GABAT and SSADH are mainly responsible for up-regulating GABA expression so as to play anticonvulsant effect [27], indicating natural GABA metabolism exerting significant control over abnormal muscle contraction.

Since the decreased GABA is a major reason of spasticity secondary to cortical disinhibition after stroke, we sought to investigate whether GABAT expression are affected by acupuncture, practically by the classic anti-spastic needling technique. In our present study, we found that the expression of GABAT was significantly increased while GABA expression decreased in the cortical infarct regions by MCAO, but acupuncture with waggle needling reversed this change, similar to the effect of baclofen, a GABA receptor agonist used for spasticity. However, routine needling at the same acupoint did not significantly attenuate GABAT expression, thus showing less therapeutic effect. Therefore, our results may promote more widespread awareness of the benefits of using suitable needling techniques in acupuncture clinical setting. In order to provide stronger evidence of the effect of waggle needling on GABAT in post-stroke spasticity, studies focusing on this effect whether could be blocked by GABAT antagonist is necessary to conduct. Moreover, whether SSADH is involved in these cascade anti-spastic effects or how the GABA receptors react still need to be further explored.

Conclusion

This study preliminary demonstrated that waggle needling wielded preferable neuroprotective and anti-spastic effects at least partly by attenuating GABAT and enhancing GABA in cortical infarct regions in an ischemia stroke model. These findings suggest that waggle needling may be a potential non pharmacological intervention for the treatment of cerebral ischemia and spasticity.

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Conflicts of interest

There are no conflicts of interest.

References

1 Pandyan AD, Gregoric M, Barnes MP, Wood D, Van Wijck F, Burridge J, *et al*. Spasticity: clinical perceptions, neurological realities and meaningful measurement. *Disabil Rehabil* 2005; **27**:2–6.
2 Li S, Francisco GE. New insights into the pathophysiology of post-stroke spasticity. *Front Hum Neurosci* 2015; **9**:192.
3 Fheodoroff K, Jacinto J, Geurts A, Molteni F, Franco JH, Santiago T, *et al*. How can we improve current practice in spastic paresis? *Eur Neurol Rev* 2016; **11**:1–8.
4 Lundström E, Smits A, Borg J, Terént A. Four-fold increase in direct costs of stroke survivors with spasticity compared with stroke survivors without spasticity: the first year after the event. *Stroke* 2010; **41**:319–324.
5 Creamer M, Cloud G, Kossmehl P, Yochelson M, Francisco GE, Ward AB, *et al*. Effect of intrathecal baclofen on pain and quality of life in poststroke spasticity. *Stroke* 2018; **49**:2129–2137.
6 Treiman DM. GABAergic mechanisms in epilepsy. *Epilepsia* 2001; **42** (Suppl 3):8–12.
7 Kowalczyk P, Kulig K. GABA system as a target for new drugs. *Curr Med Chem* 2014; **21**:3294–3309.
8 Yue ZH, Li L, Chang XR, Jiang JM, Chen LL, Zhu XS. [Comparative study on effects between electroacupuncture and acupuncture for spastic paralysis after stroke]. *Zhongguo Zhen Jiu* 2012; **32**:582–586.
9 Jayakumar AR, Sujatha R, Paul V, Asokan C, Govindasamy S, Jayakumar R. Role of nitric oxide on GABA, glutamic acid, activities of GABA-T and GAD in rat brain cerebral cortex. *Brain Res* 1999; **837**:229–235.
10 Liu C, Li R, Song X, Feng X. Effect of catgut implantation at acupoints on GABA(B) and mGluR1 expressions in brain stem of rats with spasticity after stroke. *J Tradit Chin Med* 2014; **34**:566–571.
11 Li RQ, Wan MY, Shi J, Wang HL, Liu FL, Liu CM, *et al*. Catgut implantation at acupoints increases the expression of glutamate aspartate transporter and glial glutamate transporter-1 in the brain of rats with spasticity after stroke. *Neural Regen Res* 2018; **13**:1013–1018.
12 Liu J, Chen L, Zhou J, Wang C, Fang J. [Efficacy on the range of motion of the lower limbs in patients of post-stroke spasmodic paralysis regulated with multi-directional stimulation technique]. *Zhongguo Zhen Jiu* 2016; **36**:1013–1017.
13 Shi GX, Yang CY, Wu MM, Guan LP, Wang LP, Liu CZ. Muscle hypertonia after permanent focal cerebral ischemia in rats: a qualitative and quantitative behavioral and electrophysiological study. *Int J Neurosci* 2013; **123**:575–581.
14 Zhu X, Hu H, Li Z, Lin R, Mao J, Chen L. Gua Lou Gui Zhi decoction attenuates post-stroke spasticity via the modulation of GABAB receptors. *Mol Med Rep* 2015; **12**:5957–5962.
15 Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987; **67**:206–207.
16 Chen CL, Chen CY, Chen HC, Wu CY, Lin KC, Hsieh YW, Shen IH. Responsiveness and minimal clinically important difference of modified Ashworth Scale in patients with stroke. *Eur J Phys Rehabil Med* 2019; **55**:754–760.
17 Mu JD, Ma LX, Wang JX, Song Y, Gan YY. Study on rat model of spasticity after stroke by using the suture-occluded method. *China J Tradit Chinese Med Pharmacy* 2019; **34**:5687–5690.
18 Shi GX, Yang XM, Liu CZ, Wang LP. Factors contributing to therapeutic effects evaluated in acupuncture clinical trials. *Trials* 2012; **13**:42.
19 Nierhaus T, Chang Y, Liu B, Shi X, Yi M, Witt CM, Pach D. Somatosensory stimulation with XNKQ acupuncture modulates functional connectivity of motor areas. *Front Neurosci* 2019; **13**:147.
20 Khan F, Amatya B, Bensmail D, Yelnik A. Non-pharmacological interventions for spasticity in adults: an overview of systematic reviews. *Ann Phys Rehabil Med* 2019; **62**:265–273.
21 Fridén J, Lieber RL. Spastic muscle cells are shorter and stiffer than normal cells. *Muscle Nerve* 2003; **27**:157–164.
22 Miczak K, Padova J. Muscle overactivity in the upper motor neuron syndrome: assessment and problem solving for complex cases: the role of physical and occupational therapy. *Phys Med Rehabil Clin N Am* 2018; **29**:529–536.
23 Han X, Zou YH, Fu CH, Tan ZJ. Clinical value and effect mechanism of Yanglingquan acupoint in treatment of stroke hemiplegia. *Jilin J Tradit Chinese Med* 2016; **36**:1050–1053.
24 Ramanathan M, Babu CS, Justin A, Shanthakumari S. Elucidation of neuroprotective role of endogenous GABA and energy metabolites middle cerebral artery occluded model in rats. *Indian J Exp Biol* 2012; **50**:391–397.
25 Gan P, Cheng JS, Ng YK, Ling EA. Role of GABA in electro-acupuncture therapy on cerebral ischemia induced by occlusion of the middle cerebral artery in rats. *Neurosci Lett* 2005; **383**:317–321.
26 Pearl PL, Parviz M, Vogel K, Schreiber J, Theodore WH, Gibson KM. Inherited disorders of gamma-aminobutyric acid metabolism and advances in ALDH5A1 mutation identification. *Dev Med Child Neurol* 2015; **57**:611–617.
27 Piplani S, Verma PK, Kumar A. Neuroinformatics analyses reveal GABAt and SSADH as major proteins involved in anticonvulsant activity of valproic acid. *Biomed Pharmacother* 2016; **81**:402–410.