Effect of auricular acupuncture on gastrointestinal motility and its relationship with vagal activity

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ABSTRACT
Background Vagus nerve stimulation is capable of regulating autonomic nerve function. In Traditional Chinese Medicine, the effect of auricular acupuncture (AA) is mediated by the vagus. This study was designed to investigate the effect of AA on gastrointestinal (GI) motility and the relationship of this effect with the vagus nerve.

Methods 50 rats were divided into five groups for observation of the effects of different types of acupuncture and influencing factors: control, AA, somatic acupuncture (SA), atropine and atropine +AA. The acupuncture points used for AA were ST (Stomach) and SI (Small intestine), while the acupuncture point used for SA was ST36. Electroacupuncture was performed for 15 min. A model of reduced GI motility was established using ethanol, and GI transit rate was used to measure GI motility. Heart rate variability (HRV) and the effect of atropine administration were investigated to study the relationship between AA and vagal activity.

Results The GI transit rate increased in both the AA and SA groups compared with control, and no significant difference was found between their effects. In addition, after atropine administration, AA was found to be ineffective in influencing the GI transit rate. In the HRV analysis, no significant differences were found in the absolute low frequency normalised units, high frequency normalised units or the low frequency/high frequency component ratio in the AA or SA groups compared with control. After administration of atropine AA still had no effect on HRV.

Conclusions The function of AA in improving GI motility is similar to that of SA, and this effect can be blocked by the presence of atropine, indicating that this effect is regulated by the vagus. However, HRV did not reflect the acupuncture-induced changes in vagal nerve function.

INTRODUCTION
The vagus nerve, an important component of the autonomic nervous system (ANS), plays an essential role in regulating visceral activity including gastrointestinal (GI) motility. Various studies have shown that electrical stimulation at certain intensities can effectively control autonomic nervous function, thereby causing therapeutic effects in, for example, epilepsy, depression and heart failure. This procedure is called ‘vagus nerve stimulation’.

However, early versions of vagus nerve stimulation were invasive procedures with inevitable side effects in clinical practice. More recently, with the development of research techniques in anatomy, the presence of the auricular branch of the vagus nerve (ABVN)—the Arnold nerve—has been confirmed. As a result, transcutaneous electrical stimulation performed at the auricle has been found to regulate the ANS effectively and to provide a safer, more comfortable and non-invasive procedure through the vagus nerve. The ABVN arises from the superior jugular ganglion of the vagus, and its peripheral branches are distributed to the skin of the auricle and to the posterior and inferior skin of the external acoustic meatus. Although the distribution of the ABVN in humans has notable variations among individuals, it is found in the cavum conchae of all subjects. Thus, the cavum concha is considered an area where the ABVN is accessible.

From the perspective of Traditional Chinese Medicine, auricular acupuncture (AA) is seen as an essential part of acupuncture theory in addition to somatic acupuncture (SA), and is often used in clinical practice accompanied by electrical stimulation of acupuncture points via thin metallic needles, a procedure known as electroacupuncture (EA). Based on the discovery of the ABVN and the principle of transcutaneous auricular electrical stimulation, the theory of ‘ear–vagus
reflex’ was proposed, and the ABVN is considered an important pathway for the mechanism of AA. Interestingly, according to the Chinese AA points map, all of the acupuncture points used for visceral diseases are located in the cavum conchae and cymba conchae (figure 1), including the HT (Heart), LU (Lung), ST (Stomach), SI (Small intestine) and LI (Large intestine) points. More specifically, in AA theory, the ST and SI acupuncture points, which are used to treat GI disease, are also located in the cavum conchae.

Gastrointestinal motility dysfunction, including diseases such as functional dyspepsia and irritable bowel syndrome, is very common. The discovery that dysfunction of the ANS could affect GI motility is therefore of great importance clinically. Numerous studies have focused on the intervention by SA (ST36) in GI motility, indicating the specific effects of SA in altering GI motility compared with non-acupuncture points and other SA points, and there is agreement that the effect of SA on GI motility is strongly associated with vagal activity. More specifically, the effect of SA is diminished by atropine-induced blockade of acetylcholine receptors. Based on analysis of heart rate variability (HRV), it has also been shown that SA can effectively enhance vagal activity.

Published reports have shown that the effects of AA and SA treatment are similar in certain diseases such as obesity, chronic low back pain and post-operative pain. Moreover, in some conditions, AA and SA might have the same therapeutic effect. In view of the effect of SA (ST36) on GI motility in particular, we decided to study the effect of AA in regulating GI motility. Because AA can directly stimulate the region where the vagus nerves are distributed and the transmission mechanism may be mediated by vagus nerves which are capable of controlling GI motility, we hypothesised that AA would promote acetylcholine release and enhance vagal activity, thus leading to therapeutic effects in the treatment of decreased GI motility. The following studies were undertaken to test this hypothesis.

METHODS
Study design
In an assessor-blind trial, 50 Sprague-Dawley rats were randomly divided into five groups: control group; AA group; SA group; atropine group; and AA+atropine group. A rat model of reduced GI motility was established by gavage of anhydrous ethanol and GI motility was investigated by measuring the GI transit rate. Analysis of the GI transit rates of the control group, AA group and SA group was used to determine whether AA and SA have the same effect on GI motility as non-acupuncture. The atropine and AA+atropine groups were set up to investigate the effect of atropine on AA-induced enhancement via inhibition of acetylcholine binding with M-type receptors in order to determine whether AA-enhanced GI motility is mediated through the release of acetylcholine. An HRV analysis was performed to study the effects of AA, SA and non-acupuncture on autonomic nervous activity by reflecting the dynamic balance between cardiovascular sympathetic and parasympathetic nerve activity.

The set-up of the models, electrical stimulation and recording of HRV were performed by one investigator (HL). Data on GI transit rate were collected and HRV parameters were analysed by Y-PW who was blind to the group allocation. Group allocation codes were not revealed until the analysis had been completed.

Animals
The 50 rats (no gender restriction, weight 220–250 g) were given free access to food and water and were maintained in a 12 : 12 h light/dark cycle (lights on at

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**Figure 1** (A) Location of auricular acupuncture points used for treating visceral diseases in clinic including LU (Lung), HT (Heart), ST (Stomach) and SI (Small intestine) points. (B) Distribution areas of auricular branch of vagus nerve in the external ear. In 14 ears the vagus was distributed to the crus of the helix in 20%, the crura of the anthelix in 9%, the anthelix in 73%, tragus in 45%, the cavum conchae in 45% and the cymba conchae in 100%.
08:00 h). The animals were kept individually in polypropylene cages (40×19×15 cm) in a temperature-controlled room (22±1°C) for 10 days. The experiment was carried out in our laboratory and each experiment lasted 2 h. After overnight fasting, 2 ml anhydrous ethanol was introduced to each rat to reduce GI motility. One hour later, each rat was given a gavage of Indian ink (1 ml/100 g, Chroma, Germany), maintained in a resting state by ether anaesthesia and then connected to an HRV recorder. The heart rate was continuously recorded for 1 h. Fifteen minutes after application of Indian ink, EA was performed for 15 min on the rats assigned to receive acupuncture treatment. Rats in the atropine+AA and atropine groups received an intraperitoneal injection of 10 mg/kg atropine 10 min before the Indian ink gavage. The rats were then killed, the gastric transit rate was measured and the HRV was analysed. Unfortunately, one rat in the atropine+AA group died during the experiment (figure 2).

Measurement of GI motility
GI motility was measured using the GI transit rate. One hour after the start of the experiment, Indian ink was orally administered to the animals. All animals were killed in 1 h, the cardia and pylorus were ligated and the entire GI tract was collected. The GI transit rate was calculated from the ratio of the distance that the Indian ink had migrated divided by the length of the intestine (from pylorus to caecum). This is a validated method of measuring GI motility.31–34

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\text{Gastrointestinal transit rate} = \frac{\text{the distance that Indian Ink had migrated}}{\text{the length of the intestine}} \times 100\%
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Measurement of autonomic nervous function
After administration of Indian ink, the rats were connected to a HRV recorder, a device widely used for biological signal acquisition (Powerlab 8S, ADInstruments, Australia). Three electrodes of the HRV recorder were connected to the upper right extremity, lower left extremity and lower right extremity. EA application could affect the acquisition of the heart rate signal, so data from the 15 min period of EA were discarded. For each animal, the HRV at 15 min pre-stimulation and during the 30 min post-stimulation period were sampled.

Overall power spectral analysis, which uses fast Fourier transform algorithms, was applied to the HRV signals and the percentage of power in each frequency sub-band was calculated. The percentage of power of the low frequency (LF) component (0.3–0.8 Hz) represents mainly sympathetic activity, and that of the high frequency (HF) component (0.8–4.0 Hz) represents parasympathetic or vagal activity.35 Since the physiological explanation of the term ‘very low frequency (VLF)’ components (<0.3 Hz) has not yet been clarified and VLF assessed from short-term recordings is regarded as a dubious measure, usage of LF and HF components are more widely accepted and were used here. LF and HF were calculated using the normalised units of LF and HF power (LFnu, HFnu), which represent the relative value of each power component in proportion to the total power minus the VLF components. The representation of LFnu and HFnu reflects the control and balance of the two components of the ANS.36 Moreover, LF/HF was computed as the ratio between the area under the curve within the frequency range of 0.3–0.8 Hz and the area under the curve within the frequency range of 0.8–4.0 Hz, which represents the sympathovagal balance.37

EA stimulation
Acupuncture points
The distribution of the vagus nerve in rats is similar to that in the human auricle and is located on the end of the crus of the helix, the cymba conchae and the cavum conchae. In this study, the ST acupuncture point

Figure 2 Study flow chart. Each rat ingested 2 ml dehydrated ethanol at 0 min. After 1 h the rats were injected with Indian ink (1 ml/100 g) and heart rate variability (HRV) recording started. The experiment lasted a total of 2 h. Atropine was used in the atropine group and the atropine + AA group before HRV recording. Rats not in the control and atropine groups were given electrical stimulation at acupuncture points.
points at the end of the crus of the helix and the SI acupuncture points of the cymba conchae were selected for AA, with a needle insertion depth of 1–2 mm (figure 3). For the SA group, the acupuncture points were the ST36 points on the lower bilateral extremity which are located slightly below the patella at a depth of 4–5 mm.

Electrical stimulation
Electrical stimulation was applied during both AA and SA by connecting the needles to an EA device (1 mA, 2 Hz, 15 min, Hanz, LH202H, China).

Statistical analysis
SPSS V.18.0 software was used for the statistical analysis. All data are presented as means and standard deviation. Independent t test of group comparisons was used for the analysis of the GI transit rate and HRV analysis. The statistical significance was set at p<0.05.

RESULTS
GI motility
As shown in figure 4, AA and SA significantly enhanced the GI transit rate compared with the control group (70.72±10.28 vs 56.49±12.09, p=0.011; 73.38±10.28 vs 56.49±12.09, p=0.003). There was no significant difference between the AA and SA groups (70.72±10.28 vs 73.38±10.28, p>0.05). After atropine administration the GI transit rate showed no difference between the AA+atropine group and the atropine group (42.93±16.68 vs 40.15±20.45, p>0.05).

Autonomic nervous function
As shown in figure 5, the HRV parameters LFnu, HFnu and LF/HF of the control, AA and SA groups were not significantly different in the pre-stimulation period (p>0.05). In the post-stimulation period, HRV parameters also did not show any difference in the SA and AA groups compared with the control group (p>0.05). After atropine was given, the HRV parameters in the pre-stimulation period in the atropine

Figure 3  (A,B) Acupuncture points located in the left ear of rats are defined as equivalent to ST (Stomach) and SI (Small intestine) points in humans. The dotted lines in rats are the distribution areas of the vagus, similar to humans.

Figure 4  Changes in gastrointestinal (GI) transit rates in the five groups. Auricular acupuncture (AA) and somatic acupuncture (SA) increased the GI transit rate compared with control, with no difference between AA and SA. After atropine, AA showed no effect.

*p < 0.05, **p < 0.01 difference from control group.
and atropine+AA groups were not different (p>0.05), but AA showed no effects on LFnu, HFnu and LF/HF compared with the atropine group (p>0.05).

**DISCUSSION**

Studies addressing the effect of AA on GI motility dysfunction in clinical practice have been widely reported, although only a few basic research studies have been conducted. Based on previous literature regarding SA treatment for GI motility, a reduced GI motility model using anhydrous ethanol was adopted for the study, which primarily leads to acute gastric mucosal injury, thereby inhibiting gastric contraction and acetylcholine release as well as inducing ANS dysfunction.

Our study revealed that, compared with the control group, SA significantly increased the GI transit rate in this rat model, confirming that stimulating ST36 can regulate GI motility. Moreover, the GI transit rate was increased in both the AA and SA groups compared with the control group, indicating that AA has a similar effect to SA on regulation of GI motility. This effect was inhibited by the presence of atropine. The preliminary experiment proved the hypothesis that electrical stimulation applied in the ABVN distribution...
region could regulate GI motility. In addition, it was found that this effect was significantly associated with vagal activity. The hypothesis of the vagovagal reflex is based on the facts that the nucleus tractus solitarius (NTS) is the primary brainstem of connecting vagal afferent input and the dorsal motor nucleus of the vagi (DMV) is the initiation site of the efferent nerves. These NTS neurons can use several different neurotransmitters to control the output from DMV cells which, in turn, control gastric function and complete the vagovagal loop. It has been proved that the NTS is labelled following horseradish peroxidase application to the internal auricular nerves. Previously supported by this anatomy, a possible association between the ear and visceral activity such as the ‘ear–lung’ reflex and the ‘ear–heart’ reflex has been hypothesised, indicating that visceral activity could be regulated by auricular vagus stimulation of the ABVN distribution region. On this basis, the ‘ear–vagus nerve’ reflex theory has been proposed in which AA regulates visceral function by the vagus nerve pathway.

Numerous studies have affirmed the regulatory effect of auricular vagus stimulation on the ANS. Generally, the HRV is a more objective indicator for this effect than heart rate or blood pressure. In our study, LFnu, HFnu and LF/HF were not significantly different in the pre-stimulation period, so it was concluded that the HRV in each group was the same. However, in the post-stimulation period, SA and AA did not enhance vagal activity compared with controls. This finding can be explained by the following observations. Although several studies have reported that the enhancement of vagal activity leads to an increase in HF when SA is used to promote GI motility, Ouyang et al found that increased HF and decreased LF/HF ratio occurred only during vagus nerve stimulation and both parameters returned to the normal level after stimulation. In addition, systematic research investigating the relationship between SA and HRV has found that SA does not affect the HRV.

From the anatomical point of view, the vagus nerve originates from the DMV; however, the vagal nerve fibres innervating GI motility arise from the rostrocaudal area of the DMV whereas the vagal fibres innervating the heart are found throughout the entire dorsal nucleus. The gastric and cardiac vagal nerve fibres therefore arise from different connecting sites. The origin of HRV is most probably a change in cardiac vagal activity but, in our studies, a change in gastric vagal activity was observed. Malik et al suggested that HRV is more likely to reflect the regulation of the cardiovascular ANS. The stimulation of the GI vagal nerve might not simultaneously induce cardiac vagal nerve stimulation. Overall, in our study neither SA nor AA affected HRV. After atropine administration, HRV was still not affected by AA.

Previous studies have supported this idea by reporting that ST36 EA did not affect atropine-induced HRV. Thus, AA might present characteristics with respect to atropine-induced HRV that are similar to those of SA.

**Study limitations**

Although our experimental results are positive, we have to acknowledge that our study has limitations and further studies are required. The mechanism of GI motility dysfunction in clinical practice is rather complicated and might involve many influencing factors including ANS dysfunction, gastric dysrhythmia and dysfunction of the secretion of brain-gut peptides. Although it was found that AA can improve GI motility in this rat model of reduced GI motility, the effect of AA in clinical practice has not yet been proved.

We observed that ethanol could reduce GI motility and decrease vagal activity, and these can be reversed by SA (as already known) and AA. After atropine administration the effect caused by AA was eliminated. Based on this, we concluded that the effect of AA on GI motility is mediated by cholinergic pathways. Although HRV did not show the increase in vagal activity caused by SA and AA, this could be explained by HRV being more likely to reflect the regulation of the cardiovascular ANS than the gastric ANS.

Our study also has further limitations. First, it has to be confirmed whether or not AA affects GI motility hyperfunction. Second, we cannot eliminate the possibility of the existence of other pathways for AA to influence GI motility such as the opioid peptide pathway or serotonin pathway; further research is needed into these topics. Third, more direct methods of observation can be used for GI motility such as electrogastrography or gastric volume. Finally, due to the absence of a sham acupuncture control, it may be questioned whether the effect seen on GI motility is specific. However, the specific effects of ST36 in GI motility have been proved in comparison with non-acupuncture points and other SA points and, in this study, we have demonstrated that AA has similar effects to ST36, so the effect we observed could not be considered as a general effect of electrical stimulation. However, further studies with sham acupuncture stimulation are planned to provide more conclusive results.

Although AA can influence GI motility in rats, the clinical effect is not yet confirmed and requires carefully designed studies in the future.

**CONCLUSIONS**

AA can regulate GI motility, which is an effect possibly mediated by the vagus nerve. This study supports the established concept of the relationship between the external ear and ABVN, as well as similar effects of AA and SA on the regulation of GI motility.
Summary points

- Vagus nerve fibres are sensory to the ear and motor to the GI tract.
- The GI tract effects of electroacupuncture are similar whether given in ear points or ST36.

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