



Increased Cerebral Blood Flow following L110 Acupuncture in Healthy Volunteers Observed with ^{99m}Tc-Ethyl Cysteine Dimer Single-Photon Emission Computed Tomography

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Background: Ischemic stroke is the second most common cause of death and a major cause of disability worldwide. Acupuncture is frequently advocated as an alternative treatment during stroke rehabilitation. **Aim:** The purpose of this study was to measure regional cerebral blood flow (rCBF) following acupuncture at LI-10 Shousanli in healthy volunteers using ^{99m}Tc-ethyl cysteine dimer (^{99m}Tc-ECD) single-photon emission computed tomography (SPECT). **Methods:** Fourteen healthy volunteers were enrolled in this study. A baseline brain SPECT was taken, and 3 months later, acupuncture was performed at LI-10 for 20 min, followed by a second SPECT. Statistical parametric mapping was used to analyze the changes in rCBF before and after acupuncture through a paired t-test. **Results:** Perfusion increased in the caudate, thalamus, hippocampus, and precuneus ($P < 0.05$) regions after acupuncture at LI-10 compared to baseline and decreased rCBF was observed in the frontal cortex, occipital cortex, and parietal regions compared to baseline. Differences between baseline and postacupuncture (PA) perfusion levels showed were highest in the hippocampus region, followed by the striatum, thalamus, and cerebellum regions. **Conclusion:** ^{99m}Tc-ECD SPECT revealed significant increases in rCBF for specific region PA at LI-10. These results provide reference control group data for future longitudinal studies of stroke patients receiving acupuncture therapy as an alternative treatment to improve motor function and aid intensive rehabilitation.

Key words: ^{99m}Tc-ethyl cysteine dimer single-photon emission computed tomography, LI-10 Shousanli, regional cerebral blood flow

INTRODUCTION

Stroke is the second-most common cause of death and disability worldwide.¹ Its incidence is increasing the level of burden and reducing the quality of life for family caregivers. Cerebral blood flow is typically highly correlated with cerebral glucose utilization rates, changing together with local glucose consumption in response to altered local functional activity.² Restoring blood flow to specific areas in the damaged hemisphere after stroke is associated with better rehabilitation outcomes.³

In 1998, the National Institutes of Health published a consensus statement regarding the efficacy of acupuncture in adult stroke rehabilitation that indicated acupuncture might be useful as an adjunct treatment, as an acceptable alternative, or could be included in a comprehensive management program.⁴ Acupuncture influences cerebral blood flow in healthy persons⁵⁻⁷ and patients with stroke.^{8,9}

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The main acupoint for acupuncture treatment for cerebral infarction or stroke is LI-10 Shousanli.¹⁰⁻¹⁴ The effects of acupuncture treatment, including LI-10 Shousanli, on cerebral blood flow have been evaluated by transcranial Doppler (TCD) ultrasonography¹⁵ and functional magnetic resonance imaging (fMRI).^{16,17} In nuclear medicine, single-photon emission computed tomography (SPECT) using ^{99m}Tc-ethyl cysteine dimer (ECD) as a tracer reflects not only perfusion but also the metabolic status of the brain tissue and may be more specific for revealing the degree of irreversible brain lesions, especially in patients with ischemic stroke.¹⁸ Lee *et al.*, reported that ^{99m}Tc-ECD SPECT revealed traditional acupuncture stimulation in patients following a stroke appears to activate perilesional or use-dependent reorganized sites.¹⁹ Jung *et al.* observed a specific increase or decrease of cerebral blood flow after Japanese style, superficial acupuncture, and electroacupuncture.⁷ However, the drawback of these studies was that few studies on cerebral blood flow in patients after stroke have used sham acupuncture or it is still unclear whether the change of level of cerebral blood flows in healthy volunteers for comparison to stroke patients.

The present study was designed as a pilot concept assessment study to examine the influence of acupuncture on cerebral blood flow in healthy volunteers using SPECT ^{99m}Tc-ECD imaging. The design was based on a comparison of the effects of postacupuncture (PA) to baseline. Our goal is to first test whether acupuncture has an influence on cerebral blood flow in healthy volunteers. Second, the preliminary results may provide a reference level for a control group, allowing it to be used for cerebral blood flow enhancement during poststroke rehabilitation or blood flow-related diseases.

MATERIALS AND METHODS

This study was approved by the ethical committees and review board of the Tri-Service General Hospital, Taipei, Taiwan (approval number: 1-107-05-161).

Subjects

Fourteen healthy subjects (seven women and seven men; mean age $\pm$ standard deviation, 63 ± 6 years) were recruited for this study. Informed written consent was obtained from all subjects. Participants underwent physical and neurological examinations and showed no signs of any neuropsychiatric disorders including drug or alcohol abuse and had not taken any medications for at least 3 months. None of the subjects had any current or past neuropsychiatric disorders, a family history of movement disorders, or any chronic conditions (e.g., high blood pressure, kidney disease, and asthma), as ascertained by a screening interview. None of the female subjects was

pregnant or intended to become pregnant during the study. None of the subjects had claustrophobia or experienced fainting during acupuncture.

Radiopharmaceutical

^{99m}Tc-ECD (Neurolite, DuPont Company, USA) SPECT was used to evaluate cerebral blood flow at baseline and at the time of subjects received acupuncture. The radiochemical purity of the final ^{99m}Tc-ECD complex was measured by thin-layer chromatography on Whatman MKC 18 plates developed with acetone and 0.5 M ammonium acetate (60:40). Radiochemical purity was calculated by comparing the peak for the ^{99m}Tc-ECD complex to the sum of all other peaks on the plate.²⁰ The ^{99m}Tc-ECD used had radiochemical purity of $>97\%$.

Image acquisition

A schematic representation of the study design is shown in Figure 1. Patients were scanned in a standardized setting: In a dimly lit room, with their eyes closed and minimal background noise. They were placed in this setting at least 30 min before the radioligand injection.

Cerebral perfusion imaging was performed on dual-head gamma cameras (Discovery NM/CT 670 and Discovery NM/CT 870DR, GE Healthcare, Waukesha, WI, USA) equipped with low-energy, fan-beam collimators. All subjects were injected intravenously with a single bolus dose of 740 MBq (20 mCi) ^{99m}Tc-ECD and subjects were left to rest for another 30 min in the quiet, dimly lit room before SPECT images were obtained. All subjects were imaged in a standardized manner (supine, dimly lit room, and low noise). The acquisition parameters for SPECT were a 128×128 matrix size and a voxel size of $4.42 \text{ mm} \times 4.42 \text{ mm} \times 4.42 \text{ mm}$ with 60 frames (45 s per frame) and a 10% symmetric energy window at 140 keV. Images were reconstructed using filtered back projection with a Butterworth filter (cutoff frequency of 0.45 cycles/pixel, power 15). Uniform Chang attenuation correction (AC) was used to compensate for photon attenuation. The reconstructed image had a matrix size of $128 \times 128 \times 32$ and a voxel size of $2.25 \text{ mm} \times 2.25 \text{ mm} \times 4.5 \text{ mm}$.

Baseline and postacupuncture image acquisition

The interval between the baseline and the second brain perfusion SPECT (PA) was 3 months. LI-10 Shousanli in the left upper extremity was chosen for stimulation. The acupuncture needling was performed by a skilled oriental doctor under aseptic conditions using stainless steel acupuncture needles (NRJ 3215B, Glory Medical Equipment and Materials Co., Ltd. New Taipei City, Taiwan) measuring 0.27 mm in diameter and 40 mm in length [Figure 1]. The insertion

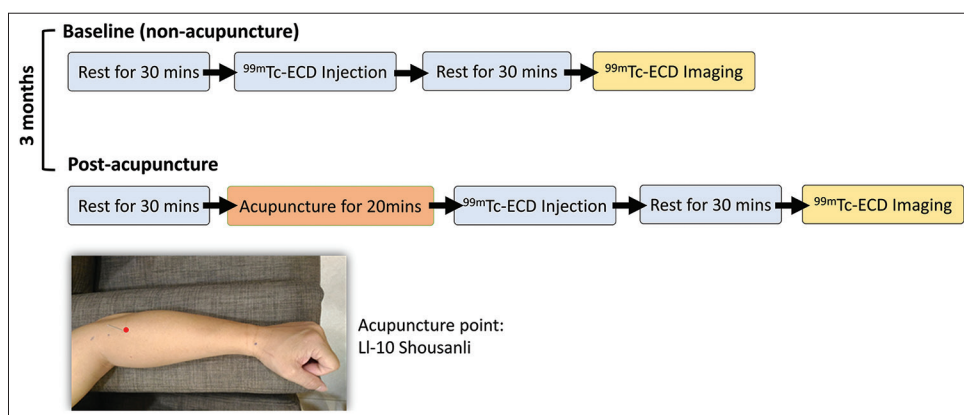


Figure 1: Schematic representation of the study design

depth of the needle into both acupoints was 2.3–2.5 cm. The needle stimulation was gentle such that subjects felt a tingling sensation and a *de qi* sensation when the needles were inserted. The needle was rotated at needle insertion, and at 5, 10, and 15 min. Needles were removed at 20 min. Subjects were then injected with ^{99m}Tc -ECD bolus intravenously for SPECT imaging.

Data analysis

Imaging data analyses

We delineated the region of interests (ROIs) using an automated anatomical labeling template (human atlas) in PNEURO (PMOD version 4.0, PMOD Technologies, Zurich, Switzerland) to prevent bias from inter-or intra-rater reliability issues arising from manual delineation. Then, the mean counts per pixel of ^{99m}Tc -ECD in the frontal cortex, temporal cortex, motor cortex, occipital cortex, caudate, thalamus, putamen, precuneus, amygdala, and cerebellum were extracted from unsmoothed standardized uptake value (SUV) images in the standard stereotactic space. The mean counts per pixel of ^{99m}Tc -ECD of the whole brain (WB) were used as the reference level.

Uptake ratio of ^{99m}Tc -ECD

The specific binding of ^{99m}Tc -ECD in selected regions was semi-quantitatively analyzed and expressed as the uptake ratio (UR). The SUV of WB was chosen as the reference level (average blood flow) for free and nonspecific binding in the brain. The UR of various ROIs, including the brain regions mentioned in the previous section, was calculated using the following equation:

$$\text{UR} = (C_{\text{ROI}} - C_{\text{WB}}) / C_{\text{WB}} \quad (1)$$

Where C_{ROI} and C_{WB} are the mean radioactivity at “pseudo” equilibrium in the ROIs and WB, respectively.

Parametric images

Images were analyzed with statistical parametric mapping software (SPM8; Wellcome Department of Cognitive

Neurology, Institute of Neurology, London, United Kingdom). All SPM calculations were performed with MATLAB version R2012a (The MathWorks Inc, Natick, MA), and the resulting voxel size was $2 \text{ mm} \times 2 \text{ mm} \times 2 \text{ mm}$. A flowchart of imaging processing and analysis is shown in Figure 2.

The average voxel values of baseline and PA image volumes were normalized to a voxel value of 100. SPM analysis (Wellcome Department of Cognitive Neurology, London, U. K.) was performed in the normal subjects for group comparison between baseline and PA. The reconstructed data, corrected for count rate, attenuation, and scatter were transformed into analyze header format: 128-pixel matrix, 35 slices, 1.9-mm pixel width and height, 4.25-mm slice thickness, and the resulting voxel size was $2 \text{ mm} \times 2 \text{ mm} \times 2 \text{ mm}$.

Each image was smoothed with a 10-mm isotropic Gaussian kernel before statistical analysis. The resulting SPM(t) maps were transformed to the unit normal distribution of SPM(z). The Z scores were computed and converted into statistical parametric maps by the software. The statistics were displayed as results and rendered on the reference three-dimensional positron emission tomography images at the threshold of $P = 0.001$ with a corrected $P = 0.05$.

Statistics

Paired *t*-tests were used to compare baseline and PA image results using GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA). *P* value less than 0.05 was considered statistically significant. All data are presented as the mean and the confidence interval (CI).

RESULTS

Parametric imaging from the voxel-wise analysis showed the cerebral blood perfusion significantly increased

after acupuncture in the anterior ventral medial prefrontal cortex, superior frontal gyrus, cingulum, insular, medial orbitofrontal gyrus, and middle cingulum [red color, two-tailed paired t -test, $P < 0.005$, Figure 3a and b]. The regions of the frontal cortex and occipital cortex showed a significant decrease in cerebral blood perfusion after acupuncture [Figure 3c].

Cerebral blood perfusion significantly increased ($P < 0.05$) after acupuncture at LI-10 in the caudate, thalamus, hippocampus, and precuneus but was significantly reduced in the frontal cortex ($P < 0.05$) and trended toward being reduced in the occipital cortex ($P = 0.0789$) and parietal region ($P = 0.0842$) [Figure 4a and b].

Comparison between baseline and poststimulation brain scans showed that the level of change in perfusion was highest in the hippocampus following by the striatum, thalamus, and cerebellum [Figure 5].

DISCUSSION

Using ^{99m}Tc -ECD SPECT, we demonstrate that cerebral blood perfusion significantly increased in the caudate, thalamus, hippocampus, and precuneus ($P < 0.05$) after acupuncture at LI-10. We also demonstrated the stimulation of LI-10 reduced the cerebral blood perfusion in the front cortex, occipital cortex, and parietal. The current results are consistent with the results of the previous study that used ^{99m}Tc -ECD SPECT⁷ or fMRI.²¹

Payabvash *et al.* (2011) reported that in acute stroke patients, the caudate body, putamen, insular ribbon, paracentral lobule, precentral, middle, and inferior frontal gyri had the highest ischemic vulnerability to hypoperfusion.²² These regions are consistent with the regions where we found a significant increase in regional cerebral blood flow (rCBF) in the current study. Moreover, we show that change levels of rCBF vary

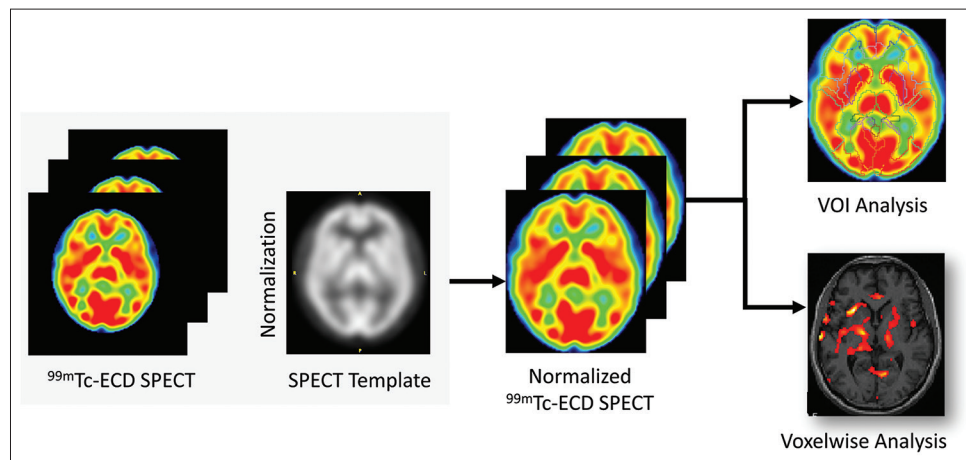


Figure 2: Flowchart of image processing and analysis methods

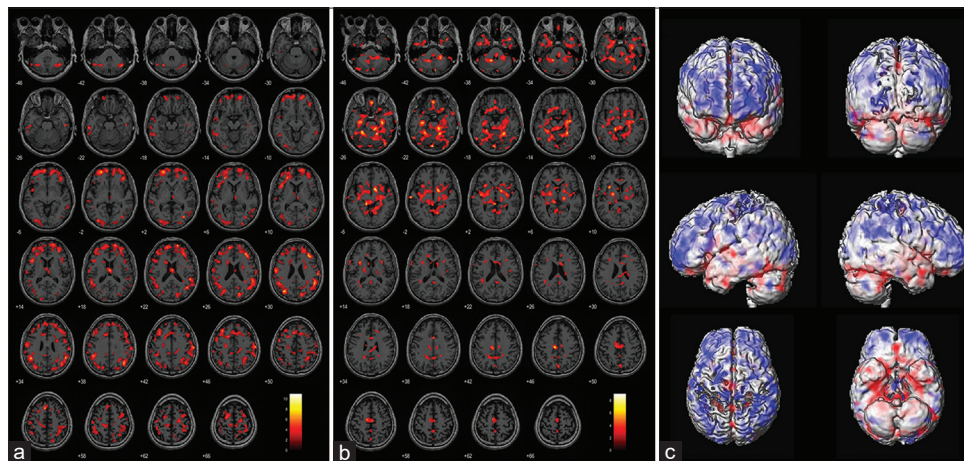


Figure 3: Brain areas (a and b) showing significant changes in perfusion after acupuncture at LI-10 (two-tailed paired t -test, uncorrected < 0.005 , extent threshold = 100, $n = 14$). Regions of increased perfusion are indicated by red and decreased perfusion is indicated by blue on the transaxial images (c)

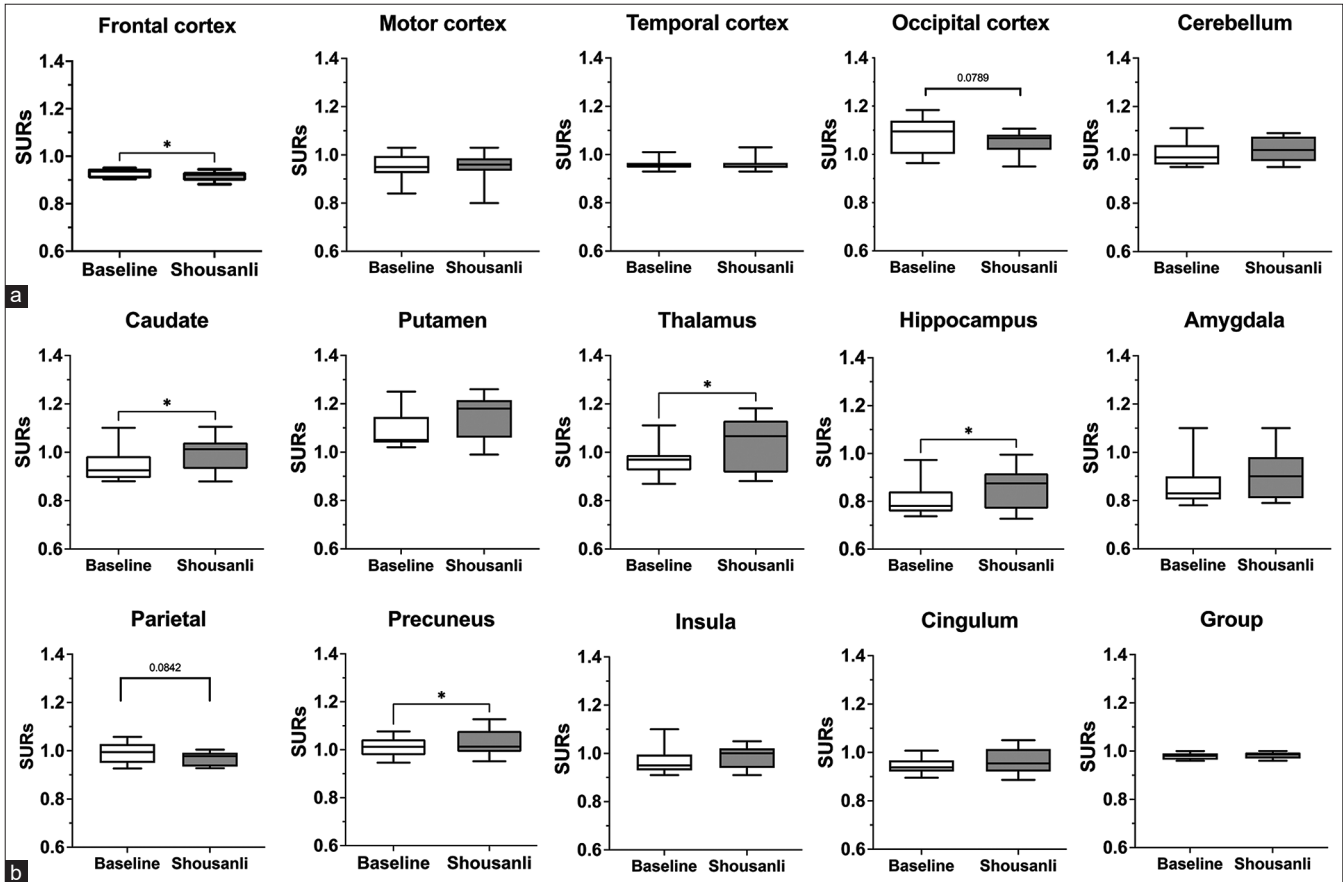


Figure 4: (a and b) Comparison of the URs of ^{99m}Tc -ECD in brain regions following acupuncture (*paired *t*-test, $P < 0.05$). ^{99m}Tc -ECD = ^{99m}Tc -ethyl cysteine dimer. URs = Uptake ratios

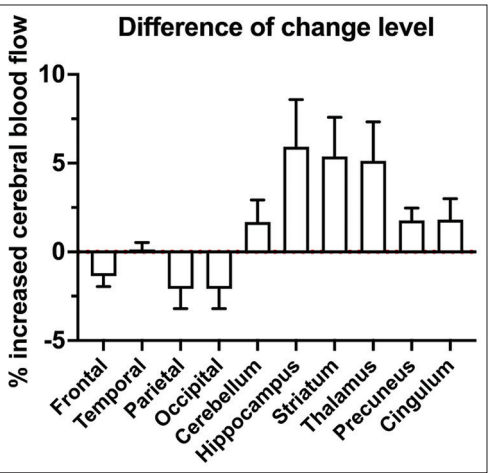


Figure 5: Change in level of blood flow in 10 brain regions between baseline and postacupuncture. None were significantly from zero

among brain regions. Thus, the current results suggest that acupuncture-induced increases in rCBF may be region-specific and associated with memory, spatial function, and emotion/mood areas.^{23,24}

We also noted a reduction in rCBF in the frontal cortex, occipital cortex, and parietal regions. An *et al.* also reported a reduction of rCBF in the above specific regions.²⁵ These results may indicate “coordination and redistribution” after stimulation.²⁶

Current results showed that both contralateral and ipsilateral cerebral regions have significant hyperperfusion PA. This suggests that both brain hemispheres were influenced by one-sided/one-acupoint stimulation. This observation may indicate that activity in the ipsilateral hemisphere induced by acupuncture is modulated through a crossed spinothalamocorticolimbic pathway through the corpus callosum.⁷

For stroke therapy, restoration of blood flow by clot lysis and reperfusion decreases ischemic injury, and this may be achievable by giving recombinant tissue plasminogen activator (tPA), the only Food and Drug Administration-approved treatment for reestablishing blood flow and salvaging brain tissue. However, tPA is used in <10% of patients and even less frequently after 3 h because of the risk of hemorrhage into ischemic tissue.²⁷ As an alternative treatment, clinical efficacy rates of

acupuncture reported in 14 articles show homogeneity in the consistency of trial results (heterogeneity: $X^2 = 8.73$, $P = 0.79$; $I^2 = 50\%$). In the combined results, the clinical efficacy rate improved significantly in acupuncture therapy (observation group) compared with the control group-baseline or healthy subjects (odds ratio = 4.04, 95% CI: 2.93–5.57, $P < 0.001$).²⁸ Clinical efficacy rate has been studied using different modalities, such as TCD, near-infrared spectroscopy, and fMRI; each provides valuable information of the brain environment and has its own limitations.

Based on these fMRI studies, these adaptive changes of the cerebral networks to brain damage could provide a basis for functional recovery.²⁹ Moreover, neuroimaging and brain mapping studies enable the study of neuronal plasticity,³⁰ which provides a critical theoretical basis for central nervous system therapy, since stroke causes not only local structural changes in the brain regions but also damage to neuronal networks, impairing sensation, movement, or cognition. Therefore, coupled imaging of SPECT/magnetic resonance imaging may provide a useful tool to for establishing mechanisms by which acupuncture activates blood flow or neurological effects.

CONCLUSION

^{99m}Tc-ECD SPECT revealed a significant increase in rCBF for specific brain regions after acupuncture at location LI-10. Both brain hemispheres were influenced by the one-sided/one-acupoint stimulation, presumably through a crossed spinothalamocorticolimbic pathway through the corpus callosum. Further studies will apply these findings as a control group for a longitudinal study for stroke patients in acupuncture therapy. Further research investigating the relationships between cerebral hemispheric changes, motor function, and intensive rehabilitation is warranted.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, C.H. Chiu, upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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