A systematic review and meta-analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke

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Acupuncture for stroke has been used in China for over 2,000 years and nowadays is increasingly practiced elsewhere in the world. However, previous studies had conflicting findings on the results of acupuncture. Here, we conducted a systematic review and meta-analysis to assess the current evidence for the effect of Baihui (GV20)-based scalp acupuncture in animal models of focal cerebral ischemia. Six databases from the inception of each database up to June 2013 were electronically searched. Primary outcomes were infarct size and neurobehavioral outcome. Ultimately, 54 studies involving 1816 animals were identified describing procedures. Meta-analysis results showed that twelve studies reported significant effects of Baihui (GV20)-based scalp acupuncture for improving infarct volume compared with middle cerebral artery occlusion group ($P < 0.01$), and thirty-two studies reported significant effects of Baihui (GV20)-based scalp acupuncture for improving the neurological function score when compared with the control group ($P < 0.01$). In conclusion, Baihui (GV20)-based scalp acupuncture could improve infarct volume and neurological function score and exert potential neuroprotective role in experimental ischemic stroke.

Acupuncture is a therapeutic form of traditional Chinese medicine (TCM) that involves the insertion of fine needles or sometimes laser on the defined points, and usually follows by stimulation using related manual or electrical techniques. Acupuncture has been used for healthcare in China and elsewhere for over 2000 years and now is still a useful medical modality for the treatment of various health problems such as stroke rehabilitation, as recommended by National Institutes of Health consensus panel. Since ancient times, stroke has been very common and is a serious neurological disorder in China. Huangdi's Internal Classic, the oldest and greatest extant classic TCM literature written by various unknown authors from the Warring States Period to the Han Dynasty (475 BC-220 AD), first recorded different stroke-related symptoms and established the theoretical basis for TCM acupuncture therapy. In modern time, acupuncture continued to be widely used for stroke because stroke remains one of the leading causes of mortality and disability worldwide and the relative poverty of effective conventional treatments, except intravenous recombinant-tissue plasminogen activator (rt-PA) within 4.5 hours after stroke onset.

Baihui (GV20) is an acupoint of the Du meridian (the government vessel), which locates at the intersection of the line connecting the apexes of the two auricles and the median line of the head, 7 cun directly above the posterior hairline and 5 cun behind the anterior hairline according to the TCM theory of acupuncture and the WHO definition. Based on the TCM theory, because Baihui is located on the highest place of the head where all the yang meridians meet, acupuncture on Baihui (GV 20) could clear the mind, lift the spirits, tonify yang, strengthen the ascending function of the spleen, eliminate interior wind, and promote resuscitation. Thus, the acupoint Baihui (GV 20) is specifically used in neurological and psychiatric diseases such as stroke, headache, dizziness, and anxiety. In fact, Baihui (GV 20) is a principle acupoint which is often selected for stroke patients. From a historical perspective, the long history of acupuncture on Baihui (GV 20) for stroke treatment can be traced back to ancient China. During Tang Dynast, Beiji Qianjin Yaofang (Essential Prescriptions Worth a Thousand Gold for Emergencies), written by Sun Simiao in 652, recorded moxibustion on Baihui (GV 20) for the stroke patients who presented with paralysis and aphasia. During Ming Dynast, Puji Fang (Prescriptions for Universal Relief), compiled by Zhu Su in 1406, used Baihui (GV 20) acupoint for various symptoms, signs and on different stages of stroke. Especially, scalp acupuncture was set up and separated from traditional acupuncture system under the influence of neuroanatomy, neurophysiology, and bioholographic principle of modern medicine in the early 1970’s. Scalp acupuncture is one of the several specialized acupuncture techniques in which a filiform needle is used to penetrate specific stimulation areas on the scalp mainly for the treatment of brain
Results

Study inclusion. We identified 5383 potentially relevant articles from six databases. After removal of duplicates, 2527 records remained. After going through the titles and abstracts, we excluded 2319 papers with at least one of following reasons: (1) case report or review; (2) not an animal research; and (3) not the researches about stroke from rigorous randomized sham-controlled trials (RCT). On comparison, systematic reviews of pre-clinical animal data could be considered if the bilateral hemispheres by assessment of Positron emission tomography (PET) in cerebral structures related to motor function on the bilateral hemispheres. Therefore, we conducted a systematic review and meta-analysis of acute focal ischemic stroke.

Studying characteristics. The 54 included studies with 1816 rats described 73 groups of comparisons based on two different outcome measures: 24 groups of comparisons including 478 rats reported data as IV, and 49 groups of comparisons using 1736 animals reported data as NIS. The rats species included Sprague-Dawley (SD) rats, Wistar rats and stroke-prone spontaneously hypertensive (SHR-SP) rats. The weight of rats varied between 180–340 g (median 240 g). Sixteen out of the 54 studies (29.6%, n = 681) were permanent middle cerebral artery occlusion (MCAO) models, while the remaining studies (n = 1135) utilized temporary MCAO models. The time of ischemia varied from 30 minutes to 3 hours. Chloral hydrate were used in 32 studies (n = 59.2%) to induce anesthesia, isolurane in 7 studies (n = 38–42, 45, 57, 71), Pentobarbital in 9 studies (n = 41, 43, 44, 46, 67, 68), urethane in 39 and thiopental in one study respectively, while no report of anesthetics in the remaining 4 studies (n = 60, 67, 79).

Eight studies performed manual acupuncture (MA)61–4, 67, 70, 77, 78 Wang EL, unpublished PhD thesis, 2008 and the rest of studies utilized EA. The selection of acupoints were as follows: 12 studies used Baihuo mono-therapy44–45, 12 studies selected Baihuo plus Shuigou18–45, Li JA, unpublished Master’s thesis, 2005; Wang C, unpublished Master’s thesis, 2005; Jin JF, unpublished PhD thesis, 2005; Luo T, unpublished Master’s thesis, 2006; Chen X, unpublished Master’s thesis, 2007; Ding J, unpublished Master’s thesis, 2007; 12 studies selected Baihuo plus Dazhui46–57, 7 studies used scalp penetration needling through Baihuo to other acupoints64–70; the rest of the 11 studies selected Baihuo plus other acupoints. Meanwhile, IV was used as the outcome measure in 24 groups (44.4%), and NIS was used in forty-nine groups (90.7%). But the standards of NIS were different, as 21 studies adopted Zea long criterion32; 13 studies used Bederson criterion33; 10 studies adopted Garcia criterion34; Kuluz35, Ludmia36, Cat37 and Sun criteria38 were cited in 1 study respectively. Sixteen studies adopted both two outcome measures. The basic characteristics of the 54 studies are shown in Table 1.

Study quality and publication bias. The median number of study quality checklist items scored was ranged from 3 to 7 out of a total 10 points. Of whom, seventeen studies got 3 points; sixteen studies got 4; ten studies got 5; six studies got 6; and five studies got 7 points (Table 2). Eight studies were online Master’s thesis or PhD thesis and not formally published; Li JA, unpublished Master’s thesis, 2005; Wang C, unpublished Master’s thesis, 2005; Jin JF, unpublished PhD thesis, 2005; Luo T, unpublished Master’s thesis, 2006; Chen X, unpublished Master’s thesis, 2007; Ding J, unpublished Master’s thesis, 2007; Wang EL, unpublished PhD thesis, 2008; Wu HY, unpublished PhD thesis, 2010. Twenty-eight studies described control temperature, including control of the room and rats anal temperature. No study described the sample size calculation. SHR-SP rats were used in one study. Random allocation to treatment group and blinded assessment of outcome were described in 49 and 15 studies respectively. No study reported cerebral ischemia was induced by an investigator who was blinded to treatment allocation. Twenty studies mentioned statement of potential conflict of interests. Twenty-four studies reported compliance with animal welfare regulations. No study used anesthetic with significant intrinsic neuroprotective activity.

Effectiveness. Twelve studies reported significant effects of BBA for improving IV compared with MCAO group (n = 172, SMD = −1.95, 95% CI: −2.80 ~ −1.10, P < 0.00001; heterogeneity χ² = 43.79, P <
0.00001, F = 75%, Figure 2); the remaining twelve studies failed to pool analysis due to the data demonstrated in the form of the TTC staining or IV percentage (%), but all of them reported the significant effects of BBA for ameliorating the IV compared with the control group (p < 0.05 or p < 0.01).

Fourteen studies showed significant effects of BBA for improving the NFS according to Zea longa criterion (n = 425, SMD = 3.93, 95% CI: −5.11 to −2.75, p < 0.00001; heterogeneity χ² = 179.39, p < 0.00001, I² = 93%, Figure 3), but the remaining seven studies did not provide raw data and thus failed for meta-analysis. NFS was significantly improved in fourteen studies in BBA group compared with control group according to Bederson criterion (n = 384, SMD = 1.51, 95% CI: −1.97 to −1.05, p < 0.00001; heterogeneity χ² = 44.90, p < 0.00001, I² = 71%, Figure 4). Four studies reported significant effects of BBA for improving NFS based on the Garcia criterion (p < 0.05 or p < 0.01), but the data were represented in the graphical form and meta-analysis was unable to be done because we failed to contact the authors. Four studies also reported the significant effects of BBA for ameliorating the NFS according to the Kuluz criteria, Ludmia criteria, Cai criteria, and Sun criteria, respectively (p < 0.05 or p < 0.01).

**Assessment of bias.** The funnel plot was roughly symmetric for the effect of BBA on IV and NFS. Thus, funnel plots did not suggest an obvious publication bias (Figure 6).

**Factors affecting the outcome measures.** In the subgroup analysis for the outcome measure according to NFS, the efficacy of acupuncture at MCAO 30 min/perfusion was better than other longer perfusion time (n = 111, SMD = −3.08, 95% CI: −3.65 to −2.51, p < 0.00001; heterogeneity χ² = 2.10, p = 0.35, I² = 5%, Figure 7A). On the other hand, BBA on MCAO 3 hours and permanent model were less effective than on MCAO 1 and 2 hours (p < 0.01). Experiments using chloral hydrate as anesthetics showed better improvement of NFS when compared with other anesthetics such as isoflurane and unreported (n = 291, SMD = −5.02, 95% CI: −6.77 to −3.28, p < 0.00001; heterogeneity χ² = 142.99, p < 0.00001, I² = 94%, Figure 7B). Compared with Wistar rats, SD rats were more sensitive to BBA treatment for improving NFS (n = 214, SMD = −1.90, 95% CI: −2.24 to −1.57, p < 0.00001; heterogeneity χ² = 9.02, p = 0.25, I² = 22%, Figure 7C). BBA treatment in the published studies was more effective than that in un-published studies (p < 0.00001, SMD = −3.51, 95% CI: −4.07 to −2.94, p < 0.00001, SMD = −2.40, 95% CI: −3.02 to −1.78; respectively, Figure 7D). In the subgroup analysis for the outcome measure according to IV, efficacy of BBA at permanent model was better than at other MCAO models (SMD = −2.86, 95% CI: −4.12 to −1.61, p < 0.00001; heterogeneity χ² = 0.95, p = 0.62, I² = 0%, Figure 8A). Experiments using chloral hydrate as anesthetics showed more reduction in IV when compared with those using isoflurane and pentobarbital anesthesia (SMD = −2.59, 95% CI: −3.95 to −1.21, P = 0.0002; heterogeneity χ² = 21.95, p = 0.0005, I² = 77%, Figure 8B). Meanwhile, experiments on SD rats gave a higher estimate of effect size in IV than that using Wistar rats (SMD = −2.14, 95% CI: −3.36 to −0.93, P = 0.0005;
| Study (years) | Species (Sex, n) | Weight | Model (method) | Anesthetic | Method to acupuncture | Outcome Index (time) | Intergroup Differences |
|--------------|-----------------|--------|----------------|-------------|------------------------|----------------------|-----------------------|
| Lu 2002<sup>34</sup> | SD Rat (male, 10/10) | 280–320 g | MCAO/2 h (tatlisumak) | 4% Isoflurane induce, 2% Isoflurane, maintain | Acupuncture 30 min/d for 5 d before operation; dispersed-dense waves of 15 Hz of frequency and current density of 1 mA. | 1.NFS (ZL, 24 h) | 1. p < 0.05 |
| Lao 2003<sup>35</sup> | SD Rat (male, 10/10) | 280–320 g | MCAO/2 h (tatlisumak) | 4% Isoflurane induce, 2% Isoflurane, maintain | Acupuncture 30 min/d for 5 d before operation; dispersed-dense waves of 2/15 Hz of frequency and current density of 1 mA. | 1. Weight, temperature, operating time | 1. p < 0.05 |
| Xiong 2003<sup>36</sup> | SD Rat (male, 10/10) | 280–320 g | MCAO/2 h (tatlisumak) | 4% Isoflurane induce, 2% Isoflurane, maintain | Acupuncture 30 min/d for 5 d before operation; 1.NFS (ZL, 24 h) | 1.p, 0.05 | 1. p < 0.05 |
| Tian 2004<sup>37</sup> | SD Rat (male, 12/12) | 250–300 g | MCAO/2 h (tatlisumak) | 2% Isoflurane, maintain | Acupuncture 30 min/d for 5 d before operation; dispersed-dense waves of 15 Hz of frequency and current density of 1 mA. | 1. IV (6 h) | 1. p < 0.05 |
| Xiong 2007<sup>38</sup> | SD Rats (male, 6/6) | 250–300 g | MCAO/2 h | 10% Chloralhydrate | Acupuncture 30 min/d for 5 d before operation; dispersed-dense waves of 4–16 Hz of frequency and current density of 1 mA. | 1. phisio parameters | 1. p < 0.05 |
| Dong 2009<sup>39</sup> | SD Rats (male, 40/40) | 280–320 g | MCAO/2 h | 1% sodium pemobarbital | Acupuncture 30 min/d for 5 d before operation; dispersed-dense waves of 15 Hz of frequency and current density of 1 mA. | 1.P-ERK1/2 | 1. p < 0.05 |
| Du 2010<sup>40</sup> | SD Rats (male, 10/10) | 280–320 g | MCAO/2 h | Sodium pentobarbital | Acupuncture preconditioning for 5 days, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min | 2.NFS (Garcia, 3 d) and IV | 2. p < 0.01 |
| Wang 2011<sup>41</sup> | SD Rats (male, 8/8) | 280–320 g | MCAO/2 h | Sodium pentobarbital | Acupuncture pretreatment for 3 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min | 2. BDNF | 2. p < 0.05 |
| Kim 2011<sup>42</sup> | SD Rats (male, 6/6) | 270–350 g | MCAO/2 h | 5% isoflurane | Acupuncture on days 4, 6, 8, 11 and 13 following MCAO, with the intensity of 3–3.5 v and frequency of 23 Hz for 5 min | 2. BDNF, 3. NR | 2. p < 0.01 |
| Wang 2012<sup>43</sup> | SD Rats (male, 10/10) | 280–320 g | MCAO/2 h | Sodium pentobarbital | Acupuncture pretreatment for 3 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min | 2. physio parameters | 2. p < 0.01 |
| Zhao 2012<sup>44</sup> | SD Rats (male, 6/6) | 280–320 g | MCAO/2 h | Sodium pentobarbital | Acupuncture pretreatment for 5 d, with the intensity of 1 mA and frequency of 2/15 Hz for 30 min | 3. a7nACHR and HMG81 | 3. p < 0.05 |
| Kim 2012<sup>45</sup> | SD Rats (male, 6/6) | NA | MCAO/2 h | 5% isoflurane | Acupuncture 5 m every 2 d for 2 weeks with bipolar waveform, 3 Hz pulses was applied for bursts of 5 s, with 2 s intervals | 2.NFS(Garcia, 1 d, 2, 3 and IV) | 2. p < 0.05 |
| Ding 2004<sup>46</sup> | SD Rats (male, 5/5) | 280–340 g | Permanent MCAO | 20 g/L Pentobarbital | Acupuncture 120 min before operation; dispersed-dense waves of 14 r/min of frequency and the intensity of stimulus was based on the slightly visible muscle twitch. | 1. IV(6 h) | 1. p < 0.05 |
| Liu 2006<sup>47</sup> | SD Rats (male, 36/36) | 250–280 g | Permanent MCAO (Bederson), | 1% Pentobarbital sodium | Acupuncture Immediately after occlusion for 30 min until sacrifice, dispersed-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on the slightly visible muscle twitch. | 1. NFS (Bederson, 3 d, 7 d, 14 d, 21 d) | 2. IV | 2. p < 0.01 |
| Study (years) | Species (Sex, n) | Weight | Model (method) | Anesthetic | Method to acupuncture | Outcome Index (time) | Intergroup Differences |
|--------------|-----------------|--------|----------------|------------|-----------------------|----------------------|------------------------|
| Shen 200748  | SD Rats (Male, 6/6) | 360 ± 20 g | MCAO/2 h (ZL) | 3% chloral hydrate (1 ml/100 g) | Acupuncture 60 min after reperfusion, disperse dense waves of 80–100 Hz of frequency and current density of 1–3 mA. | 1. NFS (Julio, 24 h) | 1. p < 0.01 |
| Li 200849    | SD Rats (Male, 8/8) | 300 ± 50 g | MCAO/1 h (ZL) | 10% chloral hydrate (350 mg/kg) | Acupuncture 30 min after reperfusion 24 h, continuous wave of 3 Hz of frequency and current density of 1–3 mA. | 1. NFS and MT count (Julio, 24 h) | 1. p < 0.01 |
| Xu 200950    | Wistar rats (Male, 18/9) | 210–290 g | Permanent MCAO (Bederson) | 100 g/L chloral hydrate (400 mg/kg) | Acupuncture immediately after occlusion 30 min (10:00 AM and 15:00PM) bid for 2 w, disperse-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on rats keeping quiet | 1. NFS (Bederson, 3 h, 14 d) | 1. p < 0.01 |
| Mu 200951    | SD Rats (Male, 10/10) | 360 ± 20 g | MCAO/2 h (ZL) | 3% chloral hydrate (1 ml/100 g) | Acupuncture after occlusion 60 min, first rotating manually right and left 0.5 min then disperse-dense waves of 80–100 Hz of frequency and current density of 1–2 mA. | 1. NFS (Julio, 24 h) | 2. p < 0.01 |
| Chen 200952  | SD Rats (Male, 18/18) | 200–250 g | MCAO/3 h (ZL) | 10% chloral hydrate | Acupuncture 15 min before occlusion 12 h and 30 min, and after occlusion every time interval of 12 h until sacrifice, first twirling, lifting and thrusting 1 min, then disperse-dense waves of 10 Hz of frequency and intensity of 2 V | 1. NFS (ZL, 12 h, 24 h, 48 h) | 2. TUNEL | 3. p < 0.01 |
| Yang 201053  | SD Rats (Male, 30/30) | 180–240 g | Permanent MCAO (Tamura) | 10% chloral hydrate (400 mg/kg) | Acupuncture 30 min/d after occlusion until sacrifice, disperse-dense waves of 5–10 Hz of frequency and the intensity of stimulus was based on rats keep quiet (3–5 V) | 1. NFS (Bederson, 2 h, 1 d, 3 d) | 2. Y-Maze test | 3. p < 0.01 |
| Cheng 201154 | SD Rats (Male, 5/5) | 300 ± 20 g | MCAO/2 h (ZL) | 10% chloral hydrate (3.5 ml/kg) | Acupuncture 30 min after reperfusion, disperse-dense waves of 2–4 mA of intensity which needle handle mild shake (1–3 V) | 1. NFS (Bederson, 2 h, 1 d, 3 d, 7 d, 21 d) | 2. SSP, CX43 | 3. p < 0.01 |
| Luo 201155   | Wistar rats (Male, 30/30) | 180–240 g | Permanent MCAO | 10% chloral hydrate (400 mg/kg) | Acupuncture 30 min/d after occlusion until sacrifice, stimulated with 1–3 mA of density and frequency of 4/20 Hz | 1. NFS (Bederson, 2 h, 1 d, 3 d, 7 d, 21 d) | 2. JAK2 mRNA, P-JAK2 protein | 3. p < 0.01 |
| Liu 201256   | SD Rats (Male, 50/50) | 180–240 g | Permanent MCAO | 10% chloral hydrate (330 mg/kg) | Acupuncture 30 min/d until sacrifice, with 1–2 mA of density and frequency of 4/20 Hz | 1. NFS (ZL, 2 h, 1 d, 3 d, 7 d, 21 d) | 2. JAK2 mRNA, P-JAK2 protein | 4. p < 0.05 |
| Kim 201357   | C57BL mice (male, 10/10) | 20–25 g | MCAO/1 h | face mask delivered isoflurane | Acupuncture 20 min/d after occlusion until sacrifice, stimulated with 1 mA of density and frequency of 2 Hz | 1. Physiological parameters | 2. p < 0.05 |
|              |                 |        |               |             |                       | 2. NFS (ZL, 24 h) and IV | 3. Cerebral perfusion | 4. p < 0.05 |
| Study (years) | Species (Sex, n) | Weight | Model (method)          | Anesthetic                  | Method to acupuncture                                                                 | Outcome Index (time)* | Intergroup Differences |
|--------------|------------------|--------|-------------------------|-----------------------------|--------------------------------------------------------------------------------------|-----------------------|------------------------|
| Chen 2000    | Wistar rats      | 230–260 g | MCAO/1.5 h (ZL)         | 10% chloralhydrate (400 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Zhou 2003    | SD Rats          | 250–270 g | MCAO/1 h (ZL)           | 1 g/kg Urethane             | Acupuncture 30 min immediately after occlusion and reperfusion respectively, disperse-dense waves of 1–1.2 mA of intensity | 1. NFS (Zl,4 d) | 1. p < 0.01 |
| Li JA (Unpublished Master’s thesis, 2005) | SD Rats (Male/ female, 28/28) | 200 ± 20 g | MCAO/30 min (ZL) | 10% chloralhydrate (300 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Wang J (Unpublished Master’s thesis, 2005) | SD Rats (Male/6,6) | 200–220 g | MCAO/1.5 h (ZL) | 7% chloralhydrate (1 ml/200 g) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Jin JF (Unpublished PhD thesis, 2005) | SD Rats (Male/ female, 30/30) | 200 ± 20 g | MCAO/30 min (ZL) | 10% chloralhydrate (300 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Luo T (Unpublished Master’s thesis, 2006) | SD Rats (Male,15/15) | 230–250 g | MCAO/30 min (ZL) | 10% chloralhydrate (300 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Chen X (Unpublished Master’s thesis, 2007) | SD Rats (Male/ female, 10/10) | 180–230 g | Permanent MCAO | 10% chloralhydrate (300 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Ding J (Unpublished Master’s thesis, 2007) | SD Rats (Male/ female, 10/10) | 180–240 g | Permanent MCAO | 10% chloralhydrate (300 mg/kg) | Acupuncture 1 h after occlusion for 7 days, stimulated with 3.5 mA of density and frequency of 100 Hz, | 1. BDNF | 1. p < 0.05 |
| Zhang 2007   | SD Rats (Male,15/5) | 230–280 g | MCAO/2 h (ZL)           | 1.5% Pentobarbital sodium (30 mg/kg) | Acupuncture 60 min before reperfusion, continuous wave of 2 Hz of frequency and current density of 3.5 mA, | 1. Infarct volume/total volume(2 h) | 1. p < 0.01 |
| Ma 2009      | SD Rats (Male,10/10) | 200 ± 20 g | permanent MCAO (Tamura), 100 mg/ L | chloralhydrate (0.35 ml/kg) | Acupuncture 20 min after occlusion 24 h, for 7 days: Baihui rotating needle 1 min, retention 20 min, Shuigou: rotating needle 1 min, retention 20 min, | 1. NFS (Bederson, 1 h, 7 d) | 2. Pathomorphism 2. NR |
| Zhou 2011    | SD Rats (Male,NA) | 250 ± 10 g | MCAO/1 h (ZL)           | chloralhydrate (400 mg/kg) | Acupuncture 30 min after occlusion, 5–20 Hz of frequency and current density of 1–1.2 mA. | 1. Blood flow | 1. p < 0.01 |
| Fu 2011      | SD Rats (Male,30/30) | 180–200 g | MCAO/1 h (ZL)           | 10% Chloralhydrate (0.3 mg/100 g) | Acupuncture 30 min after occlusion for 15 days, continuous wave of 2 Hz of frequency and current density of 1 mA., | 1. NFS(Bederson, 1 h, 15 d) | 1. p < 0.05 |
| Zhang 2006   | Wistar rats (male 32/32,) | 300 ± 20 g | Permanent MCAO (Koizumi) | 10% Chloralhydrate (0.3 ml/100 g) | Acupuncture 30 min after occlusion 6 h for 10days, rotating needles 1 min (200 times/min), repeat twice, retention 30 min (PTP taiyang), then continue to the next period of treatment; rotating needles quickly 1 min, retention 30 min (PTP qubin) | 1. NFS (Bederson, d, 3, d, 5, d, 10 d) | 2. Pathomorphism 2. p < 0.05 |
| Li 2007      | Wistar rats (male 33/33) | 200–280 g | Permanent MCAO         | 10% Chloralhydrate (35 mg/kg) | Acupuncture 30 min after occlusion 6 h for 28 days (6 days a course of treatment, and 1 day interval),then continue to the next period of treatment; rotating needles quickly 1 min, retention 30 min (PTP qubin) | 1. NFS (Bederson, 24 h, 7 d, 14 d,28 d) | 2. NFmRNA 2. p < 0.05 |
| Study (years) | Species [Sex,n] | Weight | Model (method) | Anesthetic | Method to acupuncture | Outcome Index (time)* | Intergroup Differences |
|--------------|----------------|--------|---------------|------------|-----------------------|-----------------------|-----------------------|
| Sun 200966 | SD Rats (Male, 16/16) | 270–300 g | MCAO/2 h (ZL) | Intubation anesthesia (no mention drug) | Acupuncture 30 min after reperfusion for 7 days, rotating needles 5 min, repeated every 5 minutes total 30 minutes, retention 30 min (PTP qubin) | 1. physio parameters 1. p > 0.05 | 2. cerebr blood flow 2. p > 0.05 |
|             |                |        |               |            |                       |                       |                       | 3. NFS (Ludmila, 1 h, 7 d) 3. p < 0.05 |
|             |                |        |               |            |                       |                       |                       | 4. infratend volume 4. p < 0.05 |
| Wang 200967 | SD Rats (Male, 30/30) | 280–300 g | MCAO/2 h (ZL) | NR | Acupuncture 30 min after reperfusion 3 h for twice a day, rotating needles 3 times (200 times per min), 5 min each time, retention 30 min (PTP qubin) | 1. NFS (ZL, 24 h, 48 h, 72 h) 1. p < 0.01 | 2. NFS-KB 2. p < 0.05 |
|             |                |        |               |            |                       |                       |                       | 2. pathomorphism 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 3. COX-2, NF-KbmRNA 3. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 4. COX-2, NF-Kb, TGF-β1 4. p < 0.01 |
| Zhang 200968 | SD Rats (Male, 30/30) | 200 ± 20 g | MCAO/1 h (ZL) | 10% Chloralhydrate (30 mg/kg) | Acupuncture 30 min after reperfusion for 3 days, disperse-dense waves and alternated frequency of 2 Hz and 100 Hz at an intensity of 2 mA (PTP qubin) | 1. NFS (bederson, 1 d, 2 d, 3 d) 1. p > 0.05 | 2. NR 2. NA |
|             |                |        |               |            |                       |                       |                       | 3. NFS-Kb, TGF-β1 3. p > 0.05 |
| Inoue 201069 | SD Rats (Male, 12/11) | NA | MCAO/2 h | thiopental (42 mg/kg) | Acupuncture 10 min after stroke onset, 2.5 Hz the voltage was 3–3.5 V | 1. NFS (bederson, 1 d, 2 d, 3 d) 1. p > 0.05 | 2. MRI 2. NA |
|             |                |        |               |            |                       |                       |                       | 2. MRI observations, IV 2. NA |
|             | SHR-SP Rats (Male, 18/15) | 270–300 g | Permanent MCAO (Koizumi) | 10% Chloralhydrate (30 mg/kg) | Acupuncture 10 min after reperfusion, 2.5 Hz the voltage was 3–3.5 V | 1. NFS (bederson, 1 d, 2 d, 3 d) 1. p > 0.05 | 2. MRI 2. NA |
|             |                |        |               |            |                       |                       |                       | 2. MRI observations, IV 2. NA |
| Zhang 201170 | Wistar rats (Male 32/32) | 300 ± 20 g | MCAO (Koizumi) | 10% Chloralhydrate (30 mg/kg) | Acupuncture 10 min after reperfusion 6 h for 10 day; rotating needles 1 min (200 times/min), repeat twice, retention 30 min (PTP qubin) | 1. NFS (bederson, 1 d, 2 d, 3 d) 1. p > 0.05 | 2. NR 2. NA |
|             |                |        |               |            |                       |                       |                       | 3. MMP-9 3. p > 0.05 |
|             |                |        |               |            |                       |                       |                       | 3. MMP-9 3. p > 0.05 |
| Wang 200371 | Wistar rats (Male, 13/13) | 250–280 g | MCAO/1.5 h (Abe), Diethyl ether induce, oxygen/nitrous oxide/isoflurane (30%/69%/1%) maintained | Acupuncture 30 min after reperfusion interval 1 day, total 30 days, disperse-dense waves of 3/20 Hz of frequency and current density of 3 mA (renzhong, baihui) | 1. NFS (ZL, 30 d) 1. p > 0.05 | 2. NR 2. NR |
|             |                |        |               |            |                       |                       |                       | 2. NR 2. NR |
| Wang 200572 | SD Rats (Male, 12/12) | 200–220 g | MCAO/1.5 h (Koizumi), 7% Chloralhydrate (1 ml/200 g) | Acupuncture 60 min after occlusion, waves of 5/20 Hz of frequency and current density of 1–4 mA (Renzhong and Baihui) | 1. NFS (ZL, 24 h) 1. p < 0.05 | 2. IV 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 2. IV 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 3. SOD, GSH-Px and MDA 3. p < 0.01 |
| Zheng 200673 | SD Rats (femial, 25/25) | 230–270 g | MCAO/2 h (Koizumi), 100 g/L Chloralhydrate (300 mg/Kg) | Acupuncture 30 min after occlusion 10 min; stimulate with 6 mA of density and frequency of 7 Hz (fengfu, baihui) | 1. IV (2 h TTC) 1. p < 0.05 | 2. IL-1β 2. p < 0.05 |
|             |                |        |               |            |                       |                       |                       | 2. IL-1β 2. p < 0.05 |
| Li 200774 | SD Rats (Male, 6/6) | 270–31 g | Permanent MCAO | 10% Chloralhydrate (0.4–0.5 ml/10 g) | Acupuncture 30 min after occlusion interval 12 h, total 6 times, disperse-dense waves of 2/30 Hz of frequency and current density of 2 mA (renzhong, baihui) | 1. NFS (ZL, 30 d) 1. p > 0.05 | 2. IV 2. p > 0.05 |
|             |                |        |               |            |                       |                       |                       | 2. NR 2. NR |
| Wang EL (Unpublished PhD thesis, 2005) | SD Rats (Male, 8/8) | 270–320 g | MCAO/2 h (ZL), 10% Chloralhydrate (330 mg/kg) | Acupuncture 2 min before operation and after reperfusion for 3 days and 30 min before sacrifice, rotating needles 3 min retention 15 min, rotating again (baihui, qubin) | 1. NFS (Bederson, 3 h, 1 d, 2 d, 3 d) 1. p < 0.01 | 2. IV 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 2. IV 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 3. pathomorphism 3. p < 0.01 |
| Peng 200975 | SD Rats (Male, 18/9) | 215–230 g | MCAO/1 h (ZL), 10% Chloralhydrate (0.36 ml/100 g) | Acupuncture 30 min after occlusion 5 min for 3 days, disperse-dense waves of 3.85 Hz/6.25 Hz (1.28 s/2.08 s) of frequency and current density of 0.8–1.0 mA (renzhong, baihui) | 1. NFS (ZL, 6 h, 12 h, 1 d, 2 d, 3 d) 1. p < 0.01 | 2. AQP4 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 2. AQP4 2. p < 0.01 |
|             |                |        |               |            |                       |                       |                       | 3. AQP4 mRNA 3. p < 0.01 |
| Study (years) | Species (Sex, n) | Weight | Model (method) | Anesthetic | Method to acupuncture | Outcome Index (time*) | Intergroup Differences |
|--------------|-----------------|--------|----------------|------------|----------------------|----------------------|------------------------|
| Peng 2010    | SD Rats (Male, 6/6) | 215–230 g | MCAO/1 h (ZL) | 10% Chloralhydrate (3.6 ml/kg) | Acupuncture 30 min after occlusion 5 min for 3 days, disperse-dense waves of 3.85 Hz/6.25 Hz (1.28 s/2.08 s) of frequency and current density of 0.8–1.0 mA (renzhong, baihui) | 1. NFS (ZL, 24 h) | 1. p < 0.01 |
| Wu HY        | SD Rats (Male, 16/16) | 200–250 g | Permanent MCAO | 10% Chloralhydrate (300 mg/kg) | Acupuncture 30 min after occlusion 1 h for 7 days, disperse-dense waves of 2–15 Hz of frequency and current density of 1 mA (baihui, qianding, shaogu, xuanli) | 1. NFS (ZL, 1 h, 7 d), 2. Nestin | 2. p < 0.01 |
| Chen 2011    | SD Rats (Male, 10/10) | 230–250 g | MCAO | 10% Chloral hydrate (30 mg/100 g) | Acupuncture 30 min after occlusion 5 h repeated in every 12 h interval, total 6 times, rotating needles 1 min, retention 30 min (dazhui, renzhong, baihui) | 1. NFS (ZL, 2 h, 1 d, 3 d), 2. IV | 2. p < 0.05 |
| Ma 2011      | SD Rats (Male, 25/25) | 180–250 g | MCAO/2 h (ZL) | NR | Acupuncture 30 min after reperfusion 90 min for 21 days, disperse dense waves of 20–100 Hz of frequency and the intensity of stimulus was localized muscle contractions were observed (renzhong, baihui) | 1. NFS (ZL, 1 d, 3 d, 7 d, 14 d, 21 d), 2. GAP-43, 3. GAP-43mRNA | 3. p < 0.01 |
| Tang 2011    | SD Rats (Male/femial, 12/12) | 220 ± 20 g | Permanent MCAO | NR | Acupuncture 30 min after occlusion for 2 weeks, disperse dense waves of 2 Hz of frequency and the intensity of 3–5 mA (fengfu, baihui) | 1. NFS (ZL, 2 w), 2. CPG15 | 2. p < 0.01 |

Note: 5-HT: 5- serotonin; a7nAChR: a 7 nicotinic acetylcholine receptors; Ach: acetylcholine; AQP4: aquaporin4; ATP: Adenosine Triphosphate; Bcl-2: B cell lymphoma-2; BDNF: brain derived neurotrophic factor; Ca2+: calcium ion; CBF: cerebral blood flow; COX2: cyclooxygenase 2; CPG15: candidate plasticity gene 15; CX43: connexin-43; DA: dopamine; d: day; δPKC: epsilon protein kinase C, EAAT2: Excitatory amino acid transporter-2; eNOS: endothelial nitric oxide synthase; EPSP: excitatory postsynaptic potential; ERK: extracellular signal-regulated kinases; ET-1: Endothelin-1; GAP-43: Growth associated protein-43; GABA: GABA; GABA: gamma-aminobutyric acid; GSH-Px: glutathione peroxidase; h: hour; HMG1: high mobility group box 1; ICAM-1: intercellular adhesion molecule 1; IL-1β: interleukin-1β; IV: intravenous volume; mAChR: muscarinic acetylcholine receptor; MCAO: middle cerebral artery occlusion; MDA: Malondialdehyd; Mg2+: magnesium ion; MMP-9: Matrix metallopeptidase 9; MT: melatonin; NE: Norepinephrine; NF: neurological function score; NO: Nitric oxide; NR: no report; PAL-1: plasminogen activator inhibitor-1; PTP: point to point; PS: population spike; TGF: transforming growth factor; rt-PA: recombinant tissue plasminogen activator; SD: Sprague-Dawley; SOD: superoxide dismutase; SSP: Synaptic Structural Parameters; time#: indicate the time to evaluate the outcomes; VECA: Vascular endothelial cell apoptosis; VEGF: Vascular endothelial growth factor; ZL: Zea Longa.
## Table 2 | Risk of bias of included studies

| Study | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | Total |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|
| Lu2002 |   ✓ | ✓  |     |     |     |     |     |     |     |       | 5     |
| Lao2003 |     |     | ✓  |     |     |     |     |     |     |       | 5     |
| Xiong2003 |   ✓ |     |     |     |     |     |     |     |     |       | 5     |
| Tian2004 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Xiong2007 |   ✓ |     |     |     |     |     |     |     |     |       | 6     |
| Dong2009 |     |     | ✓  |     |     |     |     |     |     |       | 6     |
| Du2010  |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Wang2011 |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Kim2011  |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Wang2012 |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Zhao2012 |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Kim2012  |     |     | ✓  |     |     |     |     |     |     |       | 7     |
| Ding2004 |     |     | ✓  |     |     |     |     |     |     |       | 6     |
| Liu2006  |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Shen2007 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Li2008   |     |     | ✓  |     |     |     |     |     |     |       | 4     |
| Xu2009   |     |     | ✓  |     |     |     |     |     |     |       | 5     |
| Mu2009   |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Chen2009 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Yang2010 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Cheng2011 |     |     | ✓  |     |     |     |     |     |     |       | 5     |
| Luo2011  |     |     | ✓  |     |     |     |     |     |     |       | 4     |
| Liu2012  |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Kim2013  |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Chen2000 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| Zhou2003 |     |     | ✓  |     |     |     |     |     |     |       | 3     |
| LiJA     |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| WangC    |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| JinJF    |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| LuoT     |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| ChenX    |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| DingJ    |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Zhang2007 |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Ma2009   |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Zhou2011 |     |     | ✓  |     |     |     |     |     |     | ✓     | 5     |
| Fu2011   |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| Zhang2006 |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Li2007   |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Sun2009  |     |     | ✓  |     |     |     |     |     |     | ✓     | 5     |
| Wang2009 |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Zhang2009 |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| Inoue2010 |     |     | ✓  |     |     |     |     |     |     | ✓     | 6     |
| Zhang2011 |     |     | ✓  |     |     |     |     |     |     | ✓     | 6     |
| Wang2003 |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| Wang2005 |     |     | ✓  |     |     |     |     |     |     | ✓     | 6     |
| Zheng2006 |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| Li2007   |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| WangEL   |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Peng2009 |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| Peng2010 |     |     | ✓  |     |     |     |     |     |     | ✓     | 4     |
| WuHY     |     |     | ✓  |     |     |     |     |     |     | ✓     | 5     |
| Chen2011 |     |     | ✓  |     |     |     |     |     |     | ✓     | 5     |
| Ma2011   |     |     | ✓  |     |     |     |     |     |     | ✓     | 3     |
| Tang2011 |     |     | ✓  |     |     |     |     |     |     | ✓     | 5     |

Note: Studies fulfilling the criteria of: (1) peer reviewed publication; (2) control of temperature; (3) random allocation to treatment or control; (4) blinded induction of ischemia; (5) blinded assessment of outcome; (6) use of anesthetic without significant intrinsic neuroprotective activity; (7) animal model (aged, diabetic, or hypertensive); (8) sample size calculation; (9) compliance with animal welfare regulations; and (10) statement of potential conflict of interests.
heterogeneity \( \chi^2 = 27.21, p = 0.0001, I^2 = 78\% \), Figure 8C). Treatment published studies also was more effective of BBA in the reduction of IV than that in un-published studies (SMD \(-2.05, 95\%\) CI: \(-2.99 \sim -1.12, p < 0.0001\); heterogeneity \( \chi^2 = 43.69, p < 0.0001, I^2 = 77\% \), Figure 8D). These results were consistent with previous subgroup analysis of NFS except time interval from the onset of ischemia.

**Discussion**

**Efficacy of BBA.** To our knowledge, this is the first systematic review and meta-analysis of English and Chinese literatures to determine the efficacy of BBA for animal model of acute ischemic stroke with IV and NFS as the outcome measures. The present study indicated that BBA could substantially improve neurobehavioral deficits and reduce infarct size in animal model of focal cerebral ischemia, suggesting that BBA have potential neuroprotective role in acute ischemic stroke.

**Methodological considerations.** This systematic review has a number of weaknesses. First, our search did not include data in other languages except Chinese and English, which may result in certain degree of selective bias. Second, negative studies are less likely to be published, and some negative results could not be obtained. In the present study, treatment in the published studies was more effective than that in un-published studies. Thus, the effect may be overestimated. Third, methodological quality of the included studies was not assessed.

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| Study or Subgroup | BBA group | Placebo group | Std. Mean Difference
|-------------------|-----------|---------------|-----------------------|
|                   | Mean | SD | Total | Mean | SD | Total | Weight | IV | Random | 95% CI | IV | Random | 95% CI |
| Chen2000          | 45.8 | 3.7 | 6     | 68.8 | 9.7 | 6     | 8.1%   | -2.89 | [-4.70, -1.08] |
| Dong2009          | 73.8 | 35.4 | 8     | 210.3 | 76 | 8     | 0.0%   | -2.11 | [-3.40, -0.81] |
| Kim2013           | 140.0 | 15.5 | 6     | 130.0 | 14 | 6     | 9.8%   | -0.61 | [-0.67, 1.90] |
| Li2007            | 40.5 | 9.7 | 5     | 71.0 | 3.65 | 5     | 6.2%   | -3.75 | [-6.20, -1.30] |
| Liu2006           | 94.0 | 9.5 | 4     | 135.1 | 13.18 | 4     | 5.9%   | -3.10 | [-5.64, -0.56] |
| Lu2002            | 125.0 | 40 | 10    | 150.0 | 45 | 10     | 11.1%  | -0.56 | [-1.46, 0.34] |
| Tian2004          | 64.2 | 13.5 | 12    | 153.4 | 18.6 | 12    | 8.0%   | -5.30 | [-7.12, -3.47] |
| Wang2003          | 0.87 | 0.3 | 13    | 1.47 | 0.31 | 13    | 11.1%  | -1.55 | [-2.44, -0.66] |
| Wang2008          | 130.2 | 40 | 5     | 192.4 | 50 | 5     | 9.4%   | -1.24 | [-2.66, 0.18] |
| Xiong2003         | 170.5 | 45.2 | 10 | 215.6 | 50 | 10 | 11.0%  | -0.91 | [-1.84, 0.02] |
| Xiong2007         | 90.0 | 15.5 | 3     | 275.2 | 27.5 | 3     | 1.5%   | -6.63 | [-13.23, -0.03] |
| Zhang2006         | 84.4 | 22.3 | 5     | 137.7 | 19.9 | 5     | 8.1%   | -2.28 | [-4.06, -0.49] |
| **Subtotal (95% CI)** | 86 | 86 | 100.0% | -1.95 | [-2.80, -1.10] |

Heterogeneity: Tau² = 1.48, Chi² = 43.79, df = 11 (P < 0.00001); I² = 75%

Test for overall effect: Z = 4.50 (P < 0.00001)

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| Study or Subgroup | BBA group | Placebo group | Std. Mean Difference
|-------------------|-----------|---------------|-----------------------|
|                   | Mean | SD | Total | Mean | SD | Total | Weight | IV | Random | 95% CI | IV | Random | 95% CI |
| Chen2009          | 1.56 | 0.58 | 18 | 2.33 | 0.52 | 18 | 8.5% | -1.37 | [-2.10, -0.63] |
| Chen2011          | 1.11 | 0.13 | 10 | 2.8 | 0.13 | 10 | 4.0% | -1.24 | [-1.64, -0.84] |
| Jin2006           | 2.01 | 0.29 | 27 | 3.02 | 0.31 | 24 | 8.4% | -3.32 | [-4.19, -2.45] |
| Lao2003           | 1.3 | 0.67 | 10 | 2.4 | 0.69 | 10 | 8.2% | -1.55 | [-2.58, -0.52] |
| Li2005            | 0.3117 | 0.1054 | 15 | 0.6893 | 0.1054 | 15 | 8.1% | -3.48 | [-4.66, -2.29] |
| Liu2006           | 0.73 | 0.35 | 15 | 2.13 | 0.69 | 15 | 8.2% | -2.49 | [-3.47, -1.51] |
| Lu2002            | 1.1 | 0.7 | 10 | 2.3 | 0.8 | 10 | 8.2% | -1.53 | [-2.55, -0.51] |
| Ma2011            | 0.6 | 0.55 | 25 | 3.4 | 0.55 | 25 | 8.1% | -5.01 | [-6.17, -3.85] |
| Peng2009          | 0.8 | 0.2 | 30 | 6.2 | 0.3 | 30 | 4.5% | -20.91 | [-24.81, -17.00] |
| Peng2010          | 1.2 | 0.2 | 6 | 5.5 | 0.2 | 6 | 1.3% | -19.89 | [-26.80, -10.90] |
| Tar2011           | 0.794 | 0.156 | 12 | 2.524 | 0.492 | 12 | 7.5% | -4.66 | [-6.31, -3.01] |
| Wu2010            | 1.31 | 0.79 | 16 | 2.44 | 0.8 | 16 | 8.5% | -1.39 | [-2.17, -0.60] |
| Xiong2003         | 1.2 | 0.63 | 10 | 2.2 | 0.79 | 10 | 8.3% | -1.34 | [-2.33, -0.36] |
| Zhang2009         | 1.31 | 0.45 | 10 | 2.05 | 0.47 | 10 | 8.2% | -1.54 | [-2.57, -0.51] |
| **Subtotal (95% CI)** | 214 | 211 | 100.0% | -3.93 | [-5.11, -2.75] |

Heterogeneity: Tau² = 4.13; Chi² = 179.39, df = 13 (P < 0.00001); I² = 93%

Test for overall effect: Z = 6.51 (P < 0.00001)

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Figure 2 | Pooled estimate of decrement in infarct volume with Baihui(GV20)-based Scalp acupuncture.

Figure 3 | Pooled estimate of improvement in neurological function score with Baihui(GV20)-based Scalp acupuncture according to Zea longa criteria.
studies was generally low, which is an inherent limitation in the primary study. Quality of a study has significant impacts on the reported outcome. For example, reporting randomization and blinding are less likely to report positive findings than those neither. Low quality of the included studies suggested that the results should be interpreted with caution.

**Implication for further studies.** There are various advantages of EA as it is more readily controlled, standardized and objectively measurable than manual acupuncture. In some situations EA was more effective than traditional acupuncture, particularly when strong, continued stimulation is required, as when treating paralysis. EA was also recommended for clinical trials and mechanism researches on acupuncture. In the present study, 46 out of 54 studies performed EA. Thus, EA have priority over Manual acupuncture on acupuncture research for the animal models of stroke.

In animal model of focal cerebral ischemia, the multitudinous pathophysiological processes which are involved in their deleterious effects over different time courses extending from the first hours to several days after vessel occlusion. In the present study, efficacy of BBA was lower in MCAO 3 hours and permanent groups compared with other temporary ischemia groups, thus it might be inferred that BBA could effectively inhibit those pathophysiological pathways preferentially activated by reperfusion. Further studies would be required to evaluate when the optimum time window for BBA treatment would close and to determine the duration of time to achieve maximum efficacy.

According to the effect size, this study indicated that SD rats recovered better than Wistar rats. A hypothesis may arise that individual genetic differences have different neuroprotective role in ischemic stroke. A comparison among anesthetics showed more effectiveness in NFS improvement in studies using chloral hydrate than studies using other two anesthetics. Thus, future study design for animal research need to select suitable anesthetics.

In the present study, most animal models of stroke are established on normotensive animals with occlusion of cerebral artery to artificially induce infarction in brain. The relevance of animal models...
Figure 6 | Bias assessment plot for the effect of Baihui(GV20)-based Scalp acupuncture on infarct volume and neurological function score.

Figure 7 | Subgroup analysis according to neurological function score (NFS). (A) The effect of the use of model on the estimate of improvement in NFS outcome; (B) The type of anesthetic on the estimate of improvement in NFS; (C) The type of strain used on the estimate of improvement in NFS; (D) The impact of published studies comparing with un-published studies on the estimate of improvement in NFS outcome. The vertical error bars represent the effect size for the individual estimates.
with normal cerebrovascular structure to human conditions remains dubious. Impressively, Scalp acupuncture had a rapid and strong effect on neurological dysfunction only in the hypertensive stroke-model by reducing the vasogenic oedema, but had no significant effects on the cytotoxic oedema, vasogenic oedema or neurological dysfunction of the MCAO rats within the time span examined. Hence, future studies need to investigate EA efficacy in animals with a co-morbidity such as hypertension, diabetes or advanced age.

**Conclusion**

In animal model of focal cerebral ischemia, BBA could improve IV and NFS. Although some factors such as study quality and possible publication bias may undermine the validity of positive findings, BBA may have potential neuroprotective role in experimental stroke.

**Methods**

**Search strategy.** We identified studies of BBA in animal models of acute ischemic stroke from Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, EMBASE, Chinese National Knowledge Infrastructure (CNKI), VIP information database, and Wanfang data Information Site. The publication time is from the inception of each database up to June 2013. The search term used was “Baihui (GV20)” in English or in Chinese. All searches were limited to studies on animals. Reference lists from the included literature were used to identify further relevant publications.

**Eligibility.** We included all controlled studies of the effect of BBA in animal models of focal cerebral ischaemia, where the outcome was measured as infarct volume (IV) or neurological function score (NFS). IV is an essential indicator of the severity of brain ischemic injury. 2,3,5-triphenyltetrazolium chloride (TTC) staining is an excellent research method that can be used to confirm the size and location of areas of infarction induced by focal cerebral ischemia in rats. Briefly, brain IV was assessed by TTC staining for 10 minutes at 37°C, followed by overnight immersion in 4% paraformaldehyde; unstained areas were defined as IV. NFS can also be useful in animal studies that evaluate the effect of new therapeutic methods; moreover, physical testing of the animals can be repeated over time and thus provide data on the evolution of the neurological deficit. However, measuring methods of NFS were inconsistent in different studies. Three neurological grading systems were most commonly used. The first grading system was published by Bederson et al. in 1986. This system consists of a scale from 0 to 3: (0) no observable deficit; (1) decreased forelimb resistance to a lateral push; (2) forelimb flexion; (3) circling behavior in addition to the former symptoms. The second system was reported by Zea Longa et al. in 1989. The scale rates the presence or absence of neurological signs in rats, and the details are as follows: 0 – no neurological deficit; 1 – retracts left forepaw when lifted by the tail; 2 – circles to the left; 3 – falls while walking; 4 – does not walk spontaneously; 5 – dead after surgery. The third system was introduced by Garcia et al. in 1995. It consists of 6 different criteria, including spontaneous activity, symmetry in the movement of the 4 limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch. The individual performance in each
test was rated on a 0 to 3 point scale. The sum of all 6 individual subscores was then calculated to give a range of 3–18. Thus, the score in healthy rats would be 18.

To prevent bias, inclusion criteria were pre-specified as follows: (1) the effect of BBA was tested on an animal model of focal cerebral ischemia induced by temporary middle cerebral artery occlusion (MCAO) or permanent MCAO; (2) IV and/or NS were compared with control animals receiving vehicle or no treatment. Pre-specified exclusion criteria were: (1) non-focal cerebral ischemia model (such as global, traumatic models, or hypoxic-ischemic models); (2) combined use of BBA and body acupuncture or ear acupuncture or any other agent with potentially neuroprotective effects; (3) no control group; (4) duplicate publications.

Data extraction. The following details were extracted from each study: (1) publication year and the first author’s name, model of ischemic stroke (transient, or permanent), and ischemic time; (2) individual data were obtained for each study, including animal number, species, sex, weight, motor impairment and scale; (3) information on treatment was obtained, including timing for initial treatment, types and method of treatment procedure, and duration of treatment; (4) outcome measures and timing for outcomes assessments were also included. IV and/or NS were statistically analyzed separately if outcomes were presented from the studies of animals at different time points, we extracted data from the last time point. If the data for meta-analysis were missing or only expressed graphically, we tried to contact the authors for further information, or calculate by ourselves if the information needed were available, or excluded the study which we could not get enough information. For each comparison, we extracted data of mean value and standard deviation from each treatment and control group of every study.

Quality assessment. We evaluated the methodological quality of the included studies by applying a ten-item modified scale32: (1) publication in a peer-reviewed journal; (2) randomization to treatment group; (3) allocation concealment; (4) blinded assessment of outcome; (5) avoidance of anaesthesia with known marked intrinsic neuroprotective properties; (7) use of animals with relevant comorbidities; (8) sample size calculation; (9) compliance with animal welfare regulations; (10) declared any potential conflict of interest. For the calculation of an aggregate quality score, each item of the ten-item modified scale was attributed one point. Two authors (WWW, XCL) independently extracted data and assessed study quality. Disagreements were solved after discussion on the details of the studies.

Statistical analysis. We considered all NSV and IV as continuous data, and then an estimate of the combined effect sizes utilizing standard mean difference (SMD) with the random effects model was given. We used the random effects model rather than the fixed effects model because heterogeneity between multi-studies has to be taken into account. Publication bias was assessed with a funnel plot23. For the assessment of heterogeneity, the I2 statistic was used.

Furthermore, to explore the impact of factors modifying on the outcome measures, we performed a stratified meta-analysis with experiments grouped according to the following: duration from onset of ischemia to treatment, anaesthetic method used, the method of assessing measures and timing for outcomes assessments were also included. IV and/or NSS were statistically analyzed separately if outcomes were presented from the studies of animals at different time points. We extracted data from the last time point. If the data for meta-analysis were missing or only expressed graphically, we tried to contact the authors for further information, or calculate by ourselves if the information needed were available, or excluded the study which we could not get enough information. For each comparison, we extracted data of mean value and standard deviation from each treatment and control group of every study.

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Author contributions

All authors have contributed to this article. W.W.W., C.L.X. and G.Q.Z. searched the databases, extracted the data, and screened trials. W.W.W., C.L.X. and L.L. reformatted the tables. G.Q.Z., W.W.W. and C.L.X. appraised the quality of included trials and drafted the full text. G.Q.Z. and L.L. were responsible for editing. G.Q.Z. also acted as an arbitrator and conceived the article. All authors reviewed the manuscript.

Additional information

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