·Basic Research·

Effects of Electro-acupuncture Preconditioning on Focal Cerebral Blood Flow in Artery and Vein of Rats with Middle Cerebral Artery Occlusion

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Abstract

Objective: To investigate the effects of electro-acupuncture preconditioning on focal cerebral blood flow (CBF) in artery and vein of rats with middle cerebral artery occlusion(MCAO), and to provide experimental evidences for primary prevention of ischemic stroke.

Method: Eighteen male Sprague-Dawley rats (two months old) were divided into 3 groups: electro-acupuncture preconditioning group (EA group), ischemia group (Is group) and Dazhui and Baihui sham group (six rats per group). The rats in the EA group were given electro-acupuncture preconditioning at Dazhui and Baihui with 2/15 density wave current for 30 minutes per day, 5 days consecutively. After preconditioning, enhanced laser speckle contrast analysis was implemented to get false color images before making middle cerebral ischemia occlusion model. Then getting false color images two hours during ischemia by laser speckle contrast analysis again.

Result: ①Relative CBF in focal cortical artery: There were significant differences at every time point in EA group and Is group ($P<0.01$, $P<0.05$) comparing with that before occlusion respectively. Compared with that before ischemia, there was significant difference at 120 min after ischemia in sham group ($P<0.05$); compared with that at 30 min after occlusion, there was significant difference at every time point in EA group from the time point 60 min after occlusion; there was significant difference at every time point in EA group and Is group ($P<0.05$) comparing with sham group; Compared with sham group, there was significant difference at 30 min and 60 min after ischemia in EA group ($P<0.05$). ② Relative CBF in focal cortical vein: There was significant difference at every time point in sham group comparing with that before occlusion ($P<0.05$); compared with that before occlusion, there was significant difference from 45 min after occlusion in EA group ($P<0.05$); compared with that before occlusion, there was significant difference from 105 min after occlusion in sham group ($P<0.05$); there was significant difference at 120 min after occlusion comparing with 60 min after occlusion in EA group; compared with sham group, there was significant difference at every time point in EA group and Is group (except 30 min after occlusion) ($P<0.05$), respectively; compared with Is group, there was significant difference at 30, 45, 60 min after occlusion in EA group ($P<0.05$). ③ The infarct brain volume detected 24 h after reperfusion in EA group was significantly smaller comparing with that in Is group ($P<0.05$).

Conclusion: Electro-acupuncture preconditioning could increase CBF velocity in artery and vein of focal cortex in rats with MCAO, which also had time-dependence. Additionally, electro-acupuncture preconditioning could decrease the infarct volume detected 24h after reperfusion, which possibly was correlated with the blood supply in ischemic penumbra.

Key word electro-acupuncture preconditioning; middle cerebral artery occlusion; cerebral blood flow

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Ischemic stroke is a common disease in clinical with high incidence, high morbidity and high mortality, which accounts for 60%—80% of stroke[1]. Moreover, the complications induced by stroke, such as movement, speech, perception, swallowing dysfunction and so on, bring much burden for patients' family and the society. Therefore, it has been the focus of attention to explore effective protective measures and study the mechanism of them.

Cerebral ischemia preconditioning[2] is the earliest discovered protective measure. Likewise, anoxic preconditioning[3] could recover the synaptic excitability in hippocampus with ischemic injury. Moreover, previous study demonstrated that[4] intermittent hyperoxia preconditioning had better protection than continuous hyperoxia preconditioning. Kapinya[5] found that isoflurane also had neuroprotective effects against cerebral ischemia. Although preconditionings mentioned above had neuroprotective effects against cerebral ischemia, they would bring impairments to patient's body, thus it's difficult for them to be accepted as protective measures for ischemia stroke in clinical.

As a health care mean accepted by the masses with broad mass base and certain dependence, electro-acupuncture preconditioning has become a research focus as a new neuroprotective means against cerebral ischemia. Studies[6] have found that electro-acupuncture preconditioning could alleviate microvascular injury caused by cerebral ischemia, mitigate cerebral edema, promote the expression of erythropoietin in ischemic perimeter [7] and reduce brain infarct volume. In addition, previous study[8] indicated that ERK1/2 maybe have a role in cerebral ischemic tolerance induced by electro-acupuncture through cannabinoid receptor-1. It worth noting that previous studies mostly paid attention to the mechanisms of cerebral ischemic tolerance at the time point 2h after ischemia, while less report investigated the mechanisms of cerebral ischemic tolerance during 2h of ischemia.

The present study monitored the cerebral blood flow (CBF) of focal cortex in artery and vein in rats with middle cerebral artery occlusion(MCAO) during 2h of ischemia using enhanced laser speckle contrast analysis so that the effects of electro-acupuncture preconditioning on cerebral local blood flow in rats with MCAO could be investigated. Meanwhile, the brain infarct volumes 24h after reperfusion were detected as morphological evidence.

1 Materials

Animals: Eighteen clean grade male sprague-dawley (SD) rats, weighing 250—280g, were provided by the Shanghai Laboratory Animal Center, Chinese Academy of Science.

Instruments: motor-operated treadmill (DSPT-202, Hangzhou Litai biotechnology company limited), Benchmark Deluxe™ cerebral stereotaxic apparatus for rats (David Kopf company), SDE-H37L dental drill (Marathon Korea), trinocular stereo microscope (XYH-05, shanghai, China), 12 bit CCD camera (Pixelfly QE, Cooke, USA), semiconductor laser diode (635 nm, Model KL5650, Forward, Shanghai, China), feedback control heating resistance pad (SS20-2,Zhenghua bio-instrument, Anhui, China).

Reagents：TTC (2, 3, 5-triphenyltetrazolium chloride), chloral hydrate.

2 Methods

2.1 Animal grouping

Eighteen rats were divided into three groups (n=6): electro-acupuncture preconditioning group (EA group), ischemia group (Is group) and sham group.

2.2 MCAO model

EA group: ①Rats were fixed to plates under waking state, then Dazhui and Baihui were chosen as acupoints (Figure 1). Specifically, Dazhui is located between the seventh cervical vertebra and the first thoracic vertebra, while Baihui is located at the intersection of the sagittal midline and the line linking the two ears.On Dazhui the depth of needle is 10 mm obliquely and on Baihui the needle is 3mm flatly to the head-end, then link the two acupoints by electro-acupuncture apparatus. The stimulation was adjusted at a current intensity till the rats' ears trembled slightly and a frequency of 2/15Hz for 30min/day, lasting for 5 days. The next day after preconditioning, the rats were anesthetized intraperitoneally with chloral hydrate (10%) at a dose of 0.36 ml/kg and fixed to a stereotaxic apparatus. The body temperature of rat was maintained at 37℃ with a heating pad putting under abdomen.②The images before ischemia were acquired by enhanced laser speckle contrast analysis as standard. A midline incision was made on the scalp and the tissues were cleaned to expose the surface of the skull with a scalpel. A 12mm*7mm window was thinned to translucency by a

Figure 1 electro-acupuncture preconditioning model

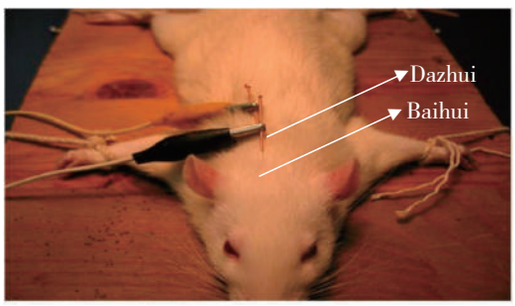
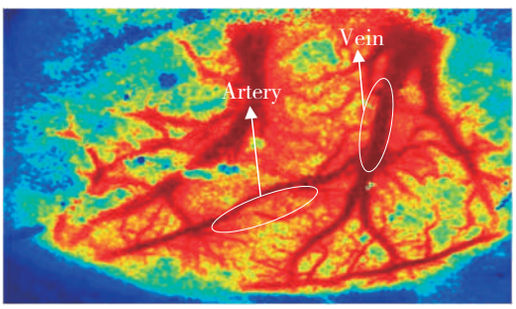


Figure 2 monitored region in experiment



high speed dental drill. The left side of the skull was placed just 10cm below the stereoscopic microscope. Then adjusted the focus to observe the cortical vascular clearly. The white light figures of focal cortical blood flow were acquired with white light irradiating the left cerebral dura mater to make sure that the region of interest was in skull window. Then the left cerebral dura mater was illuminated by a semiconductor laser diode (635nm). The laser speckle images (696*512 pixels) were acquired at 23 fps (exposure time) with a 12 bit CCD camera mounted on a trinocular stereo microscope over the thinned skull. ③The rat model of MCAO was conducted according to Longa method with some modifications. ④ The images during ischemia were acquired by laser speckle imaging after successful model. The rats with MCAO model were placed just 10cm below the stereoscopic microscope once again. The left side of exposed cerebral dura mater was illuminated by a semiconductor laser diode (635nm). The laser speckle images (696*512 pixels) were acquired at 23 fps (exposure time) with a 12 bit CCD camera mounted on a trinocular stereo microscope over the thinned skull. The false color blood flow images included relative CBF in focal cortical arteries, veins, capillaries and skull window at 30, 45, 60, 75, 90, 105 and 120min after ischemia. Monitored region in experiment was showed in figure 2.

Ischemia group: the same process as EA group without electro-acupuncture preconditioning.

Sham group: the same process as EA group without occlusion.

2.3 Determination of brain infarct volume

At 24h after reperfusion, animals were decapitated with excessive chloral hydrate. Brains were stored at -20℃ for 10min, then five coronal sections (2.5mm thick) were dissected beginning mid-way between the anterior pole and the optic chiasm and proceeding caudally. All sections were immediately placed into a 2% TTC (2, 3, 5-triphenyltetrazolium chloride) solution at 37℃ away from light with tin foil for 30min, then fixed in 4% paraformaldehyde buffer. After 24 hours, slices were photographed with a digital camera (DC240; Kodak, USA) and imaging software (Adobe Photoshop 7.0) was used to calculate the area of infarction. The total infarction volume was measured as the sum of the infarcts of each of the six sections.

2.4 Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 13.0, $P<0.05$ means statistical significance and represent for measurement data. Neurological behavioral scores and infarct volumes in ischemic rats with or without electro-acupuncture pretreatment were compared by an independent t-test. The differences of glutamate concentration in single time point and relative image density among three groups were analyzed using one-way analysis of variance (ANOVA) in single time point. Tukey's post hoc test was used to determine pairwise comparisons. Data were presented as median (interquartile range) for ordinal data and means±SD for continuous variables. All statistical assessments were two-sided and evaluated at the 0.05 level of significance.

3 Results

False color images of blood flow in EA group, Is group and sham group at different time points.

3.1 Relative local artery blood flow

It was showed in figure 3 and table 1.

3.2 Relative CBF in focal vein

It was showed in table 2.

3.3 Infarct volumes of the three groups

Infarct volumes 24h after ischemia were analyzed by one-factor analysis of variance. As seen in figure 4, infarct volume of the Is group was the biggest, while that of the EA group was lower and there was no ischemic infarct volume in sham group (P<0.01), that of the EA group was smaller than that of Is group (P<0.01), which suggested that EA could significantly decrease the ischemia infarct volume.

Figure 3 false color blood flow image of the three groups at different time point

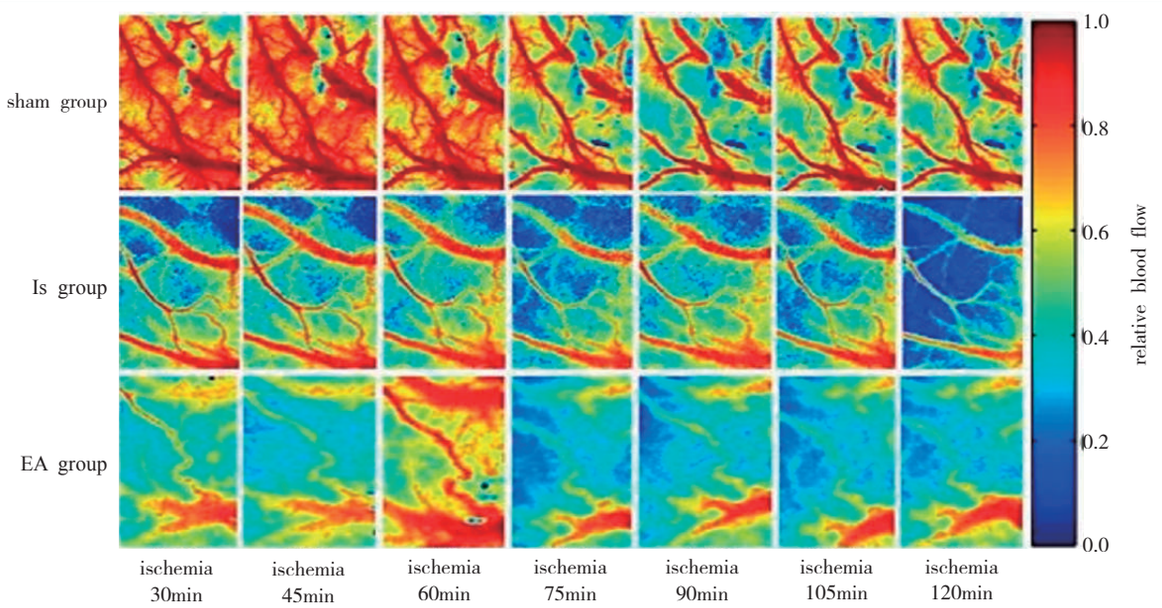


Table 1 relative local artery blood flow of the three groups at different time point (means±SD)

Group	N	Ischemia						
		30min	45min	60min	75min	90min	105min	120min
Ischemia	6	0.33±0.08	0.34±0.06	0.37±0.06	0.42±0.11	0.39±0.10	0.39±0.08	0.39±0.05
Sham	6	0.98±0.17①	0.97±0.16①	0.95±0.14①	0.90±0.17①	0.87±0.15①	0.82±0.15①	0.79±0.17①
EA	6	0.77±0.15①②	0.53±0.16②	0.56±0.13①②③	0.54±0.16②③	0.46±0.12②③	0.51±0.11②③	0.41±0.12②③

Relative velocity of CBF in three groups before ischemia is 1, standard deviation is 0, which is not listed. ①P<0.05, compared with Is group, ②P<0.05, compared EA group with sham group, ③P<0.05, compared with time point of 30 min after ischemia.

Table 2 relative velocity of CBF in focal vein of the three groups at different time point (means±SD)

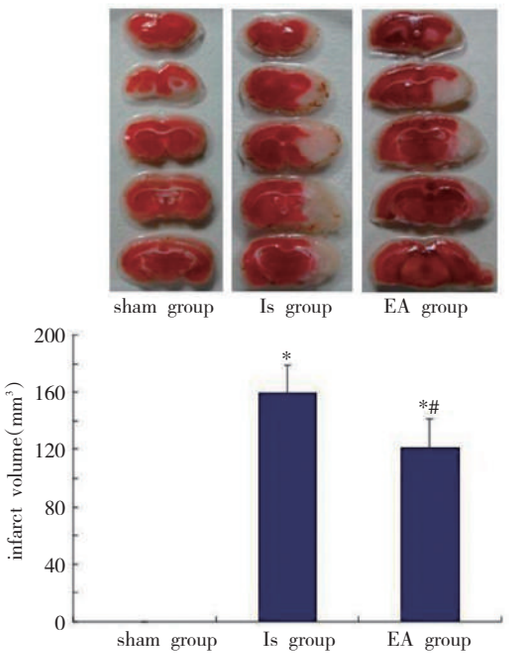
Group	N	Ischemia						
		30min	45min	60min	75min	90min	105min	120min
Ischemia	6	0.39±0.05	0.39±0.06	0.39±0.06	0.45±0.08	0.43±0.07	0.45±0.07	0.39±0.08
Sham	6	1.00±0.13①	1.02±0.16①	0.97±0.20①	0.93±0.19①	0.88±0.17①	0.80±0.14①	0.76±0.17①
EA	6	0.93±0.19①	0.68±0.14①②	0.67±0.11①②	0.63±0.11②	0.61±0.12②	0.61±0.13②	0.54±0.09②③

Relative velocity of CBF in focal vein of the three groups before ischemia is 1, standard deviation is 0, which is not listed. ①P<0.05, compared with Is group, ②P<0.05, compared EA group with sham group, ③P<0.05, compared with time point of 60 min after ischemia.

4 Discussion

It's known that brain is an organ with high oxygen consumption and low energy reserve capacity. After ischemic stroke onset, first of all, focal artery blood supply of brain tissue reduced or even the blood perfusion was totally broken off. Consequently, the pathologic processes showed up, such as dysfunction in energy metabolism, neurotoxic effects caused by glutamate, injuries induced

Figure 4 infarct volume of the three groups



*P<0.01, compared with sham group, #P<0.01, compared EA group with Is group.

by oxygen radicals and so on. The pathological processes then lead to plenty of death in nerve cells and formed reversible ischemic penumbra and nonreversible necrotic area, posing great threat to the health and life of the patients. Therefore, on the one side, doing research on the cerebral blood flow is beneficial for us to recognize the pathological process of cerebrovascular disorder and provide guidance for clinical diagnosis and treatment, on the other side, doing this research could provide fundamental support for various measures recovering blood supply in ischemic penumbra.

4.1 Two characteristics of cerebral ischemia tolerance induced by electro-acupuncture preconditioning

4.1.1 There existed two phases in cerebral ischemia tolerance induced by electro-acupuncture preconditioning: immediate effect and delayed effect. Previous study found that electro-acupuncture preconditioning for 30 minutes could induce two-phase of protective effects on focal cerebral ischemia. In detail, immediate effect emerged 2 hours after electro-acupuncture preconditioning while delayed effect emerged 24 hours after preconditioning and brain infarct volumes was significantly smaller comparing with control group[9]. Especially, delayed effect would emerge and maintain for days or weeks[10]. Chen Zebin[11]took shenshu and Baihui as acupoints and found that there existed significant difference in surviving neurons between three groups if reperfusion happened 0.5h, 1.5h and 3h after electro-acupuncture preconditioning, respectively. That is, the amount of neuronal death in group of 0.5h after preconditioning was notably less than that in group of 1.5h after preconditioning while group of 3h after preconditioning almost has no difference.

4.1.2 Electro-acupuncture preconditioning is intensity-dependent: Lao Ning[12]notified that giving electro-acupuncture preconditioning at Baihui and making the density wave current at 2/5Hz, 2/15Hz and 2/100Hz respectively, with the same stimulation time and current intensity could all induce cerebral ischemia tolerance in rats in a certain degree. However, the density of 2/15Hz exhibited the best effect.

4.2 Possible mechanisms of cerebral ischemia tolerance induced by electro-acupuncture preconditioning

The mechanisms of cerebral ischemia tolerance induced by electro-acupuncture preconditioning remain unclear and the main ones in summary are as follows: ① Electro-acupuncture preconditioning could increase the expression of hypoxia inducible factor (HIF-1α), which could prevent brain tissue from compensatory response cause by cerebral ischemia[13]. ② Electro-acupuncture preconditioning could heighten the content of adenosine (ADO), which would make the K-ATP channel open early or strong so as to inhibit the Ca^{2+} inflow and the release of excitatory amino acids and then stable the neuron membrane potentials, reduce the energy metabolism of brain and induce the cerebral ischemia tolerance to oxygen and energy supply[14]. ③Electro-acupuncture preconditioning could increase the expression of heat shock protein (HSP 70), which suggested that cerebral ischemia tolerance induced by electro-acupuncture preconditioning probably was related with the increased expression of HSP 70 around the ischemic area in cerebral cortex[15]. ④ Electro-acupuncture preconditioning could up-regulate the expression of c-fos mRNA and c-fos protein in hippocampus CA1 region, which presumed that cerebral ischemia tolerance induced by electro-acupuncture preconditioning probably correlated with the increased expression of c-fos mRNA and c-fos protein in hippocampus CA1 region[16].

⑤ The decreased content of inflammatory cytokines and the interruption of inflammatory cascade maybe correlated with cerebral ischemia tolerance induced by electro-acupuncture preconditioning[17]. ⑥ Opioid receptor might have a role in cerebral ischemia tolerance induced by electro-acupuncture preconditioning[18].

4.3 The effects of electro-acupuncture preconditioning on cerebral blod flow

Domestic research demonstrated that electro-acupuncture preconditioning could ameliorate supply of focal cerebral blood flow swiftly and alleviate brain injuries[19]. In addition, electro-acupuncture preconditioning could improve the CBF changes in rats with middle cerebral artery occlusion[20] and decrease infarct volume[21]. Meanwhile, it was found that the improvement of ischemic state induced by electro-acupuncture preconditioning possessed acupoints specificity[22].

The same kind of studies overseas discovered that electro-acupuncture preconditioning could not only ameliorate cerebral blood circulation so as to alleviate cerebral injury[23], but also promote the homeostatic equilibrium[24] and neuroregulation[25]. Moreover, electro-acupuncture preconditioning could not only increase the bilateral CBF in middle artery[26], but also increase the blood supply of contralateral hemisphere cortex and thalamus. Furthermore, Hsieh[27] found that whether cerebral hemisphere was ischemic or not, electro-acupuncture preconditioning could augment the blood flow monitored by laser-Doppler.

4.4 Findings of the present study

The present study detected cerebral blood flow by laser speckle analysis, which is high resolution, direct-view and could detect consecutively. The findings are as follows: ① The relative velocity of CBF significantly reduced after ischemia compared with that before ischemia in Is group (P<0.01). Moreover, relative velocity of CBF in focal artery has reduced to 0.33+0.08 just at 30min after ischemia. However, there was no statistical significance in relative velocity among all the time points after ischemia (P<0.05), which illustrated that relative velocity remained stable during the seven time points after ischemia. ② There was significant difference in relative velocity of CBF in focal artery among all the time points after ischemia comparing with that before ischemia in EA group (P<0.05). Additionally, relative velocity reduced to 0.77+0.15 just at 30 min after ischemia and there was statistical significance in relative velocity from the time point 60 min after ischemia compared with that 30 min after ischemia (P<0.05), which demonstrated that the relative velocity possessed downward trend at 60 min after ischemia and time points followed by in EA group. ③ There was statistical significance in relative velocity at all time points after ischemia in EA group and Is group comparing with sham group (P<0.05), which clarified that after the middle cerebral artery occlusion, relative velocity of CBF in focal artery could all reduced apparently, whether in EA group or Is group. ④ There was statistical significance in relative velocity at 30 min and 60 min after ischemia in EA group comparing with Is group, which is 1.51 and 2.33 times of Is group respectively. The statistic illustrated that electro-acupuncture preconditioning could elevate focal cerebral blood perfusion at 30 min and 60 min so as to make it possible to provide more blood supply for cerebral cortex dominated by focal artery.

Conclusions can be drawn from the data analysis of focal blood flow as follows: First, the blood flow of focal artery and the dominated cortex in EA group and Is group during ischemia remained stable. Second, the blood flow of focal artery and the dominated cortex in EA group and Is group during ischemia reduced apparently. Third, electro-acupuncture preconditioning could improve the blood perfusion of focal artery, but with time dependence.

It's known to all that various kinds of metabolic products, including inflammatory mediators, lactic acid and so on, would be generated after cerebral ischemia onset, which if being removed promptly would diminish cerebral injury. In addition, removing the metabolic products depends on the venous reflux. Besides, the present research only concerned a part of cranial window, so it couldn't reflect changes of CBF in the whole cranial window. Likewise, Conclusions can be drawn from the data analysis of CBF in focal vein as follows: ①The relative velocity significantly reduced at every time point after ischemia comparing with that before ischemia in Is group (P<0.01). Moreover, relative velocity of CBF in focal vein reduced to 0.39+0.05 just at 30 min after ischemia. However, there was no statistical significance in relative

velocity among all the time points after ischemia ($P<0.05$), which illustrated that relative velocity of CBF in focal vein remained stable during the seven time points after ischemia. There existed significant difference in relative vein blood flow between all the time points after ischemia comparing with that before ischemia ($P<0.05$). ② There was statistical significance in relative velocity from 45min after ischemia comparing with that before ischemia ($P<0.05$). Moreover, relative velocity of CBF in focal vein reduced to 0.68+0.14 just at 45 min after ischemia. However, the results were statistically significant only when comparing time points 120min after ischemia with 60min after ischemia. All of the above demonstrated that the downtrend of relative velocity was not so apparent at 45min after ischemia and from that on. ③ There was significant decrease in relative velocity at all time points after ischemia in EA group and Is group comparing with that in sham group, except 30 min after ischemia ($P<0.05$), which clarified that after the middle cerebral artery occlusion, relative velocity of CBF in focal vein could all reduced apparently at the six time points mentioned above, whether in EA group or Is group. ④ There was statistical significance in relative velocity at 30, 45 and 60 min after ischemia in EA group comparing with that in Is group ($P<0.05$), which is 2.38, 1.74 and 1.72 times of Is group respectively. The statistics illustrated that electro-acupuncture preconditioning could elevate CBF perfusion in focal vein at 30, 45 and 60 min.

We can draw some primary conclusions from the data analysis of CBF velocity in focal vein as follows: First, the CBF velocity in focal vein in Is group during the ischemic time points remained stable. Second, the CBF velocity in focal vein of EA group and Is group during the ischemic time points, except 30 min after ischemia, reduced apparently. Third, electro-acupuncture preconditioning could improve the CBF perfusion in focal vein and with time dependence as the same.

In summary, cerebral blood flow totally reduced in Is group and remained relatively stable. Besides, electro-acupuncture preconditioning could promote blood supply in a certain period of time after ischemia. Last but not the least, there existed changes of CBF in sham group which possibly was related with anesthetic.

5 Conclusion

Electro-acupuncture preconditioning could elevate the relative velocity of CBF in focal artery and vein with time dependency. The increase of relative velocity in artery maybe correlated with the enhancement of contraction rhythm in focal artery and the dominated capillaries induced by electro-acupuncture preconditioning, while the increase of relative velocity in vein maybe correlate with the enhancement of metabolic products absorption in vein induced by electro-acupuncture preconditioning. Furthermore, the decreased infarct volume 24h after reperfusion induced by electro-acupuncture preconditioning maybe related with the blood supply recovery in cerebral ischemic penumbra.

Acknowledgements

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