The effects of scalp acupuncture to the GLUT3mRNA expression of the ischemic half-dark band of MCAO model rats

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Abstract: Objiective: to study the possible mechanism of the therapeutic effectiveness of acupuncture to the cerebral injury of MCAO model rats in terms of energy metabolism through observing the effects of scalp acupincture to the GLUT3mRNA expression of the half-dark band. Methods: Choosing the heathy male Wister rats, weighed 300±20g, aged 4-5 months, making models of MCAO by means of thread-fasten method and observing the effects of acupuncture to the GLUT3mRNA expression of half-dark band. Results: Acupuncture can raise the level of GLUT3mRNA expression of half-band tissues, which indicates that there's correlation between acupuncture's protective effects to the ischemic half-darlband and the elevation of the GLUT3mRNA expression.

Keywords: Acupuncture therapy, MCAO model rats, GLUT3

Preface: In the previous work, we systematically studied the therapeutic effects of scalp acupuncture via replicating the model rats who were made cerebral focal ischemia in their right brains by means of thread-fasten method. The results showed that acupuncture apparently reduced the cerebral infarction volume of MCAO rats($p<0.05$) and effectively accelerated their recovery from neural function lost. However, we still do not know through what mechanism the effectiveness works, which will be studied in this experiment by observing the GLUT3mRNA expression of the half-dark band.

1 Materials and techniques:

1.1Instruments and reagents: KDW808Ⅱelectrical all-around inpulse curing set; low-temprature speeding centrifuge; PCR set; digital gel imaging analysis system; RT-PCR box

1.2 Fabrication techniques of MCAO model[1]: Picking single-itemed nylon threads of diameter of 0.2mm, heating one end so that the end gets spherical whose diameter is 0.25mm around. Then, cutting into segments of 20±0.5mm. These threads should be dealt with in the alcohol solution of 75%.Healthy male Wister rats which weighd 300±20g, aged 4~5 months provided by Experimental annimal center of Heilongjiang university of TCM, were received abdominal injective anesthesia by 0.5ml/100mg urethane solution of 20%. After fixing the rats supinely, operating on the prepared skin of the rats' neck. Cutting on the neck away from the middle line for 0.5cm right-bound, making the cut horizontally 2cm around. Separating the muscles and trachea hierarchically, then separating the right arteria carotid communis from the basis and putting a 3-0 type suture under the artery, separating the internal and external carotid artery on the upper thyroid , fastenning the external carotid artery and its branches with 3-0 type suture; Preparing a thread at the nearer end of internal carotid artery while clamping a artery pin at the further end; Fastenning the right arteria carotis communis and making a small cut at the crotch through which the prepared nylon thread's spherical end going into the inlet of the artery inside the cerebrum(19±0.5mm around), during this process we may get a little resistance and find that the rats' right iris becoming paler. After that, knitting the thread at the nearer end and making sure that the internal carotid artery was completely choked and the thread was fixed steadily; Then, stitching the cut up hierarchically and scattering some sulfanilamide on this area. Marking the rats with picric acid and feeding them under the same condition after having done all the surgery.After the surgery, the rats that get paralysis on the left side, especially the left limbs. Pulling their tails up in the air , the left limbs would coil and flex or over-extend passively, at the same time, turn around anticlockwise and tumble.

1.3 Experimental procedures

1.3.1 Grouping: Choosing 130 male Wister rats, grouping 3 stochastically: 30 for the group of fake surgery (Group FS) and grouping the 30 into 5 groups---6h, 24h, 48h, 72h and 7d, 6 rats for each group ; 50 for the group of MCAO (Group M) and grouping the 50 into 5 groups ---6h, 24h, 48h, 72h and 7d, 10 rats for each group. 50 for the group of acupuncture (Group A) and grouping the 50 into 5 groups---6h, 24h, 48, 72h and 7d, 10 rats for each group.Group FS: Only with nylon threads thrusted into the jugular artery for 6mm, not inside the brain, and not given any treatment after the surgery.Group M: Not given any treatment after the MCAO fabrication surgery in their right cerebrums.Group A: After the MCAO fabrication surgery, not needling the rats until they become lucid, doing the acupuncture after every 12 hours. The group of 6h only being needled once while the groups of 24h, 48h, 72h and 7d being needled twice per day. All the rats being put to death respectively at the appointed time.

1.3.2Acupuncture method: Fixing the Group A with the rats' heads normally fur-shaved and disinfected, choosing the filiform needles of 0.19mm×10mm. The first needle-thrusting from the very middle of the rat's skull to the right outer front of pinna. The second, paralell to the first one, in front of it for 3mm. After the needling, connecting the electrical curing set with frequency 7HZ, intensity 6mA, 30min remained.

1.3.3 Parameters of study

1.3.3.1 All of the rats being decapitated in the limited time, getting out the cerebrums on the ice-based utensils as quickly as possible and dumping the cerebellum, olfactory and brain stem, then slicing each cerebrum coronarily into 4 which are all 3mm in thickness. Getting 100mg approximately cortical tissues from the cortex of the second slice and perserving in liquid nitrogen. Measuring the GLUT3mRNA transcription level by RT-PCR method.

1.3.3.2 RT-PCR operation procedures:

RNA extraction: Taking 100mg cortical tissues and grinding them into powder in a mortar with liquid nitrogen; Then moving them into a homogenizer and homogenizing with 1ml Trizol reagent; Shifting the composite into a new centrifugal tube of 1.5ml in which the composite got whisked with the needle of 5ml and succeeding to cultivate on ice bed for 5min; Centrifugating at 4℃ 10000rpm/min for 15min before moving the supernatant into a new centrifugl tube of 1.5ml adddig into 0.2 ml choloroform and shaking the tube for 15 seconds before cultivating on ice bed for 5min; Centrifugating again at 4℃ 10000rpm/min for 10min and moving the supernatant into a new centrifugal tube of 1.5ml; Adding into 0.5ml isopropyl alcohol and shaking the tube to make it homogeneous before cultivating on ice bed for 5min; Then centrifugating at 4℃ 10000rpm/min for 5min and dumping the supernatant; Adding 1ml alcohol of 75% into the sediment and shaking the tube for a while; Centrifugating at 4℃ 7500rpm/min for 5min and dumping the supernatant again, making it transparent at 25℃; Then adding into 25μl DEPC solution to dissolve the RNA and perserving it at -70℃ in a low-temprature refrigerator; Measuring its OD; Then starting the RNA denaturation electrophoresis and observing the bands of 18s and 28s; Testing the 260/280 light absorption level ratio with spectrophotometer and calculating the quantity of extracted RNA meanwhile keeping the A260/A280 ratio over 1.8;

RNA revers transcription:

The following primer being designed in the reference⑵ by GLUT3cDNA turn: 5'-CGAGAGTCCAAGGTTCTTGC-3'upstream,5'-ACTGGAGGACAACGGAGATG-3'downstream, length 215bp. B-actin as the inner reference, the primer sequence: 5'-GTGCCCATCTACGAGGGTTA-3'upstream,5'-TCTCAGCTGTGGTGGTGAAG-3'downstream,length 130bp.Getting 1ug model RNA above and reverse-transcribing into cDNA.

20μg reaction system:

25mmol/L Mgcl2 4μl
10×buffer 2μl
10mmol/L dNTP 2μl
Rnasin 0.5μl

Reverse transcriptase 0.7μl
Oligo primer 1μl
RNA 1μl

Adding into distilled water without Rnase reaching to 20μl

Reaction condition: Compound cDNA cultivated at 42℃ for 45min;Reverse transcriptase deactivated at 95℃ for 10min; The cDNA substance perserved at -70℃

PCR: Amplifying in vitro according to the cDNA by PCR.

50μg reaction system:

Original cDNA 5μl
10×buffer 5μl
10mmol/L dNTP 4μl
Taq enzyme 1μl
Upstream and downstream primer 1μl

Adding double-distilled water reaching to 50μl

Reaction condition: Pre-denaturation: 94℃ for 5min; Denaturation: 94℃ for 30sec; Heating off: 55℃ for 60sec; Extension: 72℃ for 60sec;Extension at 72℃ for 5min after finishing 30 circulations; Amplified substance perserved at 4℃

RT-PCR substance analysis: Concocting 300ml buffer solution of 0.5×TBE; Colloid concentration 1.7%(40ml 0.5×TBE with 0.68g colloid); The colliod dissolved in a microwave for 2min; Adding into 2ul ethidium bromide(terminal concentration of 10mg/ml 0.5μg/ml); After its getting cool, puuting into comb and waiting for the condensation; Adding into 8μl PCR post-reaction substance + 2μl bromphenol; Electrophoresis for 3min at 100v, observing in ultra-violet light and taking photos.Testing the electrophoresis band density of PCR substance by the digital gel imaging analysis system and calculating the relative quantity of GLUT3 substance.

Formula:

Relative quantity of GLUT3 = electrophoresis band density / β-actin electrophoresis band density ×100%

1.4Statistical analysis Each group's statistics presented by average figure±standard deviation, the analysis including a t-testing with the SPSS11.5 software.

2 Outcome

Table 1: the GLUT3mRNA expression of ischemic half-dark band cortex at various time of each group (x±s)

Groups	rats	6h	24h	48h	72h	7d
Group FS	30	0.32±0.01	0.31±0.04	0.30±0.01	0.31±0.01	0.31±0.04
Group M	45	0.34±0.08	0.59±0.09**	0.51±0.04**	0.45±0.05*	0.32±0.05
Group A	45	0.71±0.04**△△	0.82±0.08**△△	0.63±0.07**△△	0.45±0.06*	0.36±0.04*

PS: ** comparing to Group FS p<0.01; △△ to Group M p<0.01; * to Group FS P<0.05

No death in Group FS at any time point during the experiment, 30 left; No death of group 6h in Group M, 10 left; 1 died in group 24h, 9 left;2 died in group 48h, 8 left; 1 died in group 72h, 9 left; 1 died in group 7d, 9 left; totally 45 left in Group M; No death of group 6h in Group A, 10 left; 1 died in group 24h, 9 left; 1 died in group 48h, 9 left; 2 died in group 72h, 8 left; 1 died in group 7d, 9 left; totally 45 left in Group A.

3 Discussion

The glucose is the main energy resources for cerebral tissues. It needs to be transferred inside the cells by GLUT in terms of facilitated diffusion because of its fat-soluble attribute. The GLUT family includes at least 7 kinds of isomers. In the cerebral tissues there mainly exists GLUT1 and GLUT3. The GLUT3 distributes in blood-brain barrier while GLUT1 only exists in the nervous system of rodent due to its rigorous tissue specificity. Both of them play important roles to the glucose transferring in the cerebral tissues. There're evidences showing that GLUT3 whose genes express in the active and energetic cells would increase its quantity with the elevating of the local glucose utilazation rate. It turns out to be evidently superior than GLUT3 on the ability of

transferring glucose. The glucose metabolism in cerebrum is mainly controlled by hexokinase under normal condition. However, the glucose's transmembrane transferring possibly is a speed-limited process of glucose utilizing when the tissues are defficient in energy. There're not many reserches regarding the GLUT1 and GLUT3 transcription level in ischemic cerebrum. Some immane grouped experiments showed that, in the situation of cerebral ischemia, the GLUT3 protein increased around the infarction spot but merely last for a short time in the early stage, which indicated that the ischemic half-band tissues were sensative to the ischemic reaction in the early stage therefore these issues managed to secure their energy supply through elevating the transcription level of GLUT3mRNA so as to boost the glucose transferring. The GLUT3 protein increasing time which began to decline and get less than the control gro3up after 24h was relatively short. This phenomenon of divergence between the protein expression and the extent of mRNA may be due to the depressing of translation function and the resisting to protein's sythesis after transcription. In the early stage of ischemia, cells improve glucos absorption and energy metabolism by boosting the GLUT3mRNA expression, which is one of the cells' protective reactions. The experiment studied the dynamic flunctuation of transcription level of GLUT3mRNA in the ischemic half-dark band of MCAO rats. The results showed: the GLUT3mRNA transcription level of half-dark band started to increase after 6h of ischmia and reached the top after 24h, and began to decline after 48h gradually.

There's still no report before regarding the effects of acupuncture to the GLUT3 transcription in half-dark band of cerebral ischemia. This experiment took the first step. It made the following conclusions: Acupuncture can elevate the GLUT3mRNA transcription level of cerebral ischemic half-dark band within 3 stages---6h, 24h, and 48h after the MCAO formed. The discrepancy between the GLUT3mRNA transcription level of cerebral ischemic half-dark band of group 6h, 24h, and 48h in Group A and that of the Group M at the corresponding time points was obvious ($p<0.01$). However, there's no obvious discrepancy between the GLUT3mRNA transcription level of group 72h, 7d in Group A and that of Group M at the corresponding time points by statistical analysis($p>0.05$). The results indicated that scalp acupuncture possibly can enhance the GLUT3mRNA transcription level in the early-suffered ischemic half-dark band tissues and boost the transferring and absorption of glucose thereby improve the energy metabolism of cerebral ischemic tissues, meanwhile secure the fundamental energy supply and protect the cerebral ischemic half-dark band from impairment. The effectiveness of scalp acupuncture to the cerebral injury of MCAO model rats may be related to acupuncture's elevating the GLUT3 transcription level of half-dark band of cerebral tissues and protecting the half-dark band. Nevertheless, the correlations, between GLUT3 and the energy metabolism of cerebral ischemia to which the acupuncture has significant effects, are extremely complicated. Therefore, these effects still need to be studied farther.

References

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