Dynamic Study of BaiHui-QuBin scalp acupuncture on Regulation of Expression of Matrix Metalloproteinase-9 in rats following middle cerebra lartery occlusion

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Abstracts :Objective : To investigate the effects of BaiHui-QuBin scalp-acupuncture on MMP-9 expression in the rats with cerebral ischemia reperfusion for exploring the interaction mechanisms. **Methods :** Focal cerebral ischemia was induced by intraluminal middle cerebral artery occlusion and reperfusion (MCAO/R) method using a monofilament thread in male SD rats. The rats of the pinprick set were acupunctured on the trouble side by BaiHui-QuBin scalp-acupuncture. We observed the brain water content of each set with the method of dry-wet weight ; detected the positive expression and protein content of MMP-9 by immunohistochemistry and western blot methods respectively in brain tissue of each set. **Results :** Compared with model group, the brain water content has been significantly reduced in acupuncturing scalp group in IR24h,IR48h,IR72h after reperfusion ($p<0.05$). In the same time phase point, compared with the model set, the pinprick set's expression ofMMP-9 was lower obviously and showed the significant difference ($P<0.05$).**Conclusion :** BaiHui-QuBin Scalpacupuncture ameliorates brain edema in rats after acute cerebral ischemia/reperfusion by possibly inhibiting MMP-9 expression.

Keywords: BaiHui-QuBin Scalp-acupuncture ; Cerebral infarction ; Cerebral ischemia/reperfusion injury ; Matrix Metalloproteinase-9.

Introduction

Recent studies have found that cerebral ischemia and reperfusion after matrix metalloproteinases (matrix metalloproteinases, MMPs) increased, particularly MMP-9 activity increased with cerebral microvascular permeability, blood-brain barrier permeability and blood-brain barrier crashes, inflammation cell infiltration and brain edema associated.In this study, we use the right middle cerebral artery occlusion rat model that study after focal cerebral ischemia and reperfusion expression of MMP-9 and by "Baihui" transparent "Qubin" Scalp therapy intervention was observed the expression of MMP-9.

Materials and methods

Experimental animals and grouping

Clean healthy male SD rats 205(weighing 280-300g) that were randomly divided into three groups: sham operation group 45, model group and acupuncture group each 80. Each group was based on ischemia reperfusion 2h at different times into IR4h, IR12h, IR24h, IR48h, IR72h five subgroups.

Screening successful animal model and model

Refer Zea Longa rat MCAO right middle cerebral artery occlusion and reperfusion model 2h. After the rats left limb paralysis, pull tail left upper limb flexion appeared vacant, or when traveling tilted to the left, circling, suggesting that successful model.

Experimental animals intervention

Scalp acupuncture group: given scalp acupuncture in awake rats after two hours of modeling, acupuncture once every 12h. Refer to "animal acupuncture points map" to take ipsilateral “Baihui” transparent "Qubin" , about 0.8 inch needle, the needle 30min, during twisting three times, each time 5min, speed 200r/min.Sham group: Before plug, only the wire tied into the 12mm, without blocking the middle cerebral artery. Sham group and model group rats after modeling, daily

treatment time without treatment, but have to crawl and fixed time (30min / times) to ensure that the rats in the acupuncture group and the same conditions.

MMP-9 immunohistochemistry

65 rats were drawn after the paraffin sections, dewaxed, PBST washing, 3% H202 solution was incubated for 5 ~ 10min; 0.0lmol / L, PH6.0 citrate buffer solution, heated to 92 ~ 98 ℃ 30min, PBS wash 5min; dropping closed with goat serum working solution, incubated for 10min, decanted, do not wash; dropping 1:50 dilution of anti-(Rabbit anti-MMP-9, CHEMICON, AB19016), 4 ℃ overnight; negative control with PBS instead of primary antibody; dropping goat anti-rabbit secondary antibody working solution at room temperature for 20min; dropping chain enzyme horseradish peroxidase-labeled avidin working solution at room temperature for 20min, each of the above steps have been thoroughly washed with PBST; DAB staining hematoxylin, dehydrated graded alcohol, xylene, neutral resin were mounted, light microscope. Positive cells was brown or tan. Using Motic Med6.0 digital image analysis system, the use of digital cameras camera after each slice in the next randomly selected 400 times around the infarct zone five non-overlapping horizons, calculate five horizons MMP-9 average number of positive cells.

MMP-9 Western blot detection

65 rats were in the corresponding time points after reperfusion brains were removed, remove the ice olfactory brain, cerebellum, lower brainstem, the separation of the ipsilateral cerebral cortex and basal ganglia, placed in vials, the -80 ℃ low temperature refrigerator. The specimens were immersed in lysis buffer sonication in an ice water bath, the supernatant after centrifugation. According to the standard curve protein The protein content of each sample. 300ug protein samples taken dissolved in buffer, 96 ℃ 5min denatured by heating sample added on 10% polyacrylamide gel and 5% into the layer of gel, electrophoresis 100V at room temperature 2h, then the gel at 4 ℃ transferred to NC membrane, soaked in TBST buffer 2h. The membrane was placed in 1:500 rabbit anti-rat MMP-9 antibody, 4 ℃ overnight incubation; TBST wash three times, each time 10min; then placed in 1:1000 dilution of rabbit anti-rat-HRP reaction 1 hours; TBST washed three times, each time 10min; finally dropping ECL film developer, dark room exposure, color print film. Determined using the analysis software Imagepro plus6.0 MMP-9 protein and internal reference protein β-Tubulin the western-blot gray ratio. It is calculated as follows: the measured intensity of the target protein × area / internal reference protein β-Tubulin intensity × area. Therefore, greater than gray, MMP-9 protein expression is higher.

Statistical

SPSS13.0 statistical software for statistical analysis, the data were presented as mean ± standard deviation (± SD) representation. Two sets of data were compared using the t test, multiple sets of data were compared using the SNK and LSD test. $P < 0.05$ difference was statistically significant.

Results

MMP-9 immunohistochemical staining results

This study found that, under optical microscope, sham-operated rats almost no MMP-9 expression in brain tissue.Model group increased expression of MMP-9, is mainly expressed in ischemic neurons, astrocytes and endothelial cell cytoplasm or membrane, where after reperfusion 24h ~ 48h MMP-9 expression is most obvious. Scalp acupuncture group also visible expression of MMP-9, but was significantly reduced compared with the model group.

Table 1 Each rat ischemic brain tissue MMP-9 positive cells compared ($\bar{x}$ ± SD)

Grouping	n	IR4h	IR12h	IR24h	IR48h	IR72h
Sham group	15	2.50±0.71	3.50±0.71	2.50±0.71	3.00±1.41	1.50±0.71
Model group	25	20.40±2.41$^{\triangle}$	26.20±3.77$^{\triangle}$	35.60±2.70$^{\triangle}$	37.40±2.88$^{\triangle}$	26.80±3.19$^{\triangle}$
Acupuncturegroup	25	15.60±3.36$^{\triangle\bigstar}$	21.20±2.59$^{\triangle\bigstar}$	26.00±3.67$^{\triangle\bigstar}$	30.20±2.58$^{\triangle\bigstar}$	22.60±2.70$^{\triangle\bigstar}$

$^{\triangle}$ $P < 0.05$ With the same point in time sham group ;

$^{\bigstar}P < 0.05$ With the same point in time model group.

MMP-9 protein expression in western blot test results

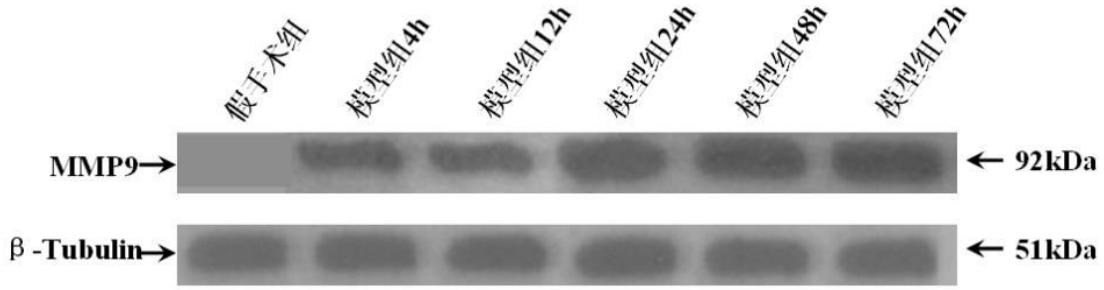

Figure 1：MMP-9 protein bands of sham and model group

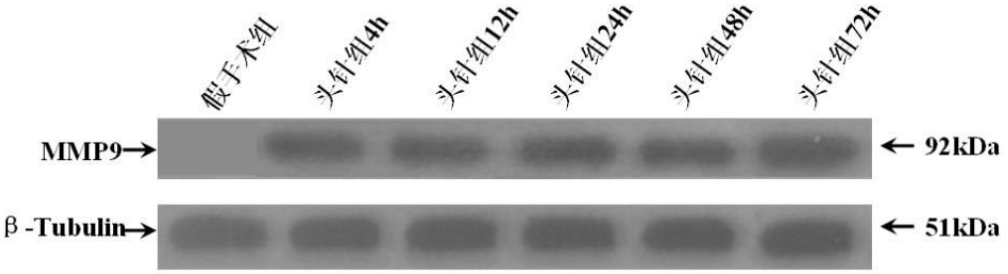

Figure 2：MMP-9 protein bands of sham and acupuncture group

Discussion

MMPs are a group containing Zn2 + dependence of Ca2 + can be degraded or modified extracellular matrix proteases[1], the normal state in zymogen form, and is controlled at a low level. In a number of physiological and pathological conditions, the activity was significantly increased in tissue damage and repair.MMPs MMP-9 is the largest family of enzymes in molecular weight, primarily by synthesis and secretion of vascular endothelial cells, astrocytes, microglia, infiltration of neutrophils and ischemic neurons can also be expressed[2].

In recent years, studies have shown that[3-4], MMP-9 involved in ischemic brain injury, and blood-brain barrier opening after reperfusion, inflammatory cell invasion and brain edema associated. Romanic, etc[5]. MCAO rat model of persistent study found 12h after stroke in the ischemic brain tissue can observe significant activity of MMP-9, 24-hour activity reached its highest value, sustained decline after 5d in the first 15 days to normal levels. Chen Yu[6] found that cerebral ischemia and reperfusion after ischemia within 6 hours MMP-9 positive cells began to appear, the first 12 hours was significantly increased, to the first two days and reached the peak, the first three days after the number of positive cells began to decrease , to 14 days to return to basal levels, each adjacent time points were significantly different.

Early cerebral ischemia, cerebral hemodynamic changes due, injury, inflammation and oxidative stress caused by factors such as MMP-9 expression and activity increases, the increase of the role of MMP-9 in the basement membrane, type IV collagen protein hydrolyzate , laminin, fibronectin and other basement membrane components, destroying the tight junctions between vascular endothelium, resulting in increased permeability of the blood brain barrier[7-8]. Blood-brain barrier permeability increase on the one hand can cause the blood plasma protein, a variety of toxic substances and metabolites into the brain, promote and aggravate vasogenic cerebral edema; the other hand, can also cause inflammatory cells invade the brain tissue and the infiltration of neutrophils themselves can be expressed MMP-9, with the ability to pass through the base film, so that the neutrophil migration in the brain, and release large amounts of oxygen free radicals, vasoactive substances, damage surviving neurons, increased tissue damage. Simultaneous overexpression of MMP-9, open the BBB and destruction, but also after cerebral ischemia and reperfusion hemorrhagic transformation is a very important reason. Therefore, MMP-9 is the process of ischemia-reperfusion injury is one of the important media, early blocking of MMP-9 expression and activation on cerebral ischemia-reperfusion injury is one effective way.

In this study, the use of immunohistochemistry, Western blot detection methods, cerebral ischemia and reperfusion was observed at different time points after MCAO / R rats with ischemic brain tissue MMP-9 protein expression was found: reperfusion after ischemia brain tissue levels of MMP-9 was as reperfusion time dynamic changes, and then began to increase perfusion 4h, 12h increased significantly, 24h ~ 48h reached the peak, followed by somewhat lower, which is observed in the experimental brain water content Changes consistent, thus proving the MMP-9 involved in the BBB after cerebral ischemia-reperfusion damage and secondary brain edema formation. Early intervention in scalp treatment, scalp rats at various time points after reperfusion ischemic brain tissue MMP-9-positive cell count and protein content were significantly reduced, with the same point in time model group , with a significant difference ($P < 0.05$) and increase of MMP-9 by excessive degradation of ECM components of the basement membrane ECM degradation leaving the normal balance is destroyed, resulting cascade after cerebral ischemia and reperfusion injury."Baihui" transparent "Qubin" scalp therapy may inhibit endogenous synthesis and secretion of MMP-9, MMP-9 induced to reduce matrix degradation, BBB open vasogenic brain edema, leukocyte infiltration and secondary brain damage, and thus play its antagonistic cerebral edema and cerebral ischemia-reperfusion injury.This is the prevention and treatment of ischemic stroke provides a new way of thinking.

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Clinical observation on nausea and vomiting caused by the chemotherapy drug cisplatin treated by“heweijiangni” Acupuncture therapy

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Abstracts: Chemotherapy is one of the important methods of malignant tumor therapy,and nausea and vomiting are the most common adverse reaction caused by chemotherapy.Patients with severe nausea and vomiting often fear and they often can't cooperate with treatment,therefore,the effectiveness of treatment often is reduced.Through of traditional Chinese medicine acupuncture