Combine Diffusion Tensor Imaging and RGMa Immunohistochemical Analysis to Evaluate the Crossed Cerebellar Diaschisis in Rats after Middle Cerebral Artery Occlusion

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Target audience
Neuroscientists, clinicians, and MRI physicists interested in stroke animal models.

Introduction Cerebral infarction can not only cause pathophysiological changes of the infarction core area, but also cause the secondary changes of the distant regions. It is believed that the mechanism of cerebral infarction might be related to the theory of crossed cerebral-cerebellar diaschisis (CCD). CCD theory pointed out that supratentorial focal brain damage could cause short functional changes in its fiber linked distant areas [1]. In this study, we try to use diffusion tensor imaging to detect the changes of diffusion parameters in remote regions of the infarct core in rats after middle cerebral artery occlusion (MACO) at a continuous time point. Meanwhile, the RGMa protein expression of the same location was detected by immunohistochemical at the same time point. The two values were analyzed to reveal the diffusion condition and pathological changes of the bilateral cerebellar hemispheres and hope to give more evidences about the relevant mechanism of crossed cerebellar diaschisis.

Method Seventy adult healthy clean level male Sprague-Dawley rats (270g~320g) were used. All the animals were randomly divided into two groups, fifty-six rats were assigned to the experimental group and fourteen rats were put in the control group. The left MACO was established by the improved approach Longa suture method. The control group and the experimental group rats were set in Signa HDxt 3.0T MR scanner (GE Healthcare, USA) with multi-channel rat coil (the Medical Science and Technology Corp. of Chenguang, Shanghai)in 1h, 3h, 6h, 9h, 12h, 24h and 72h after stroke onset, thus seven time points were obtained and eight rats were examined at each time point. Single-shot DTI images were acquired with following parameters: TR/TE＝2500/92.2ms, FOV=110×110 mm^2, NEX=4.00, matrix 128x128, b = 0,500 s/mm^2, 15 directions. After scan, the RGMa protein expression in the bilateral cerebellar hemispheres of the subjects were detected by immunohistochemical methods. FA values in the ROI which was performed in the bilateral cerebellar hemispheres were measured in each time point. The expression intensity of RGMa protein was recorded too. Paired t-test was used in statistical analysis between the control group and the experimental group at each time point for FA value and expression intensity of RGMa protein at a significant level <0.05. And the correlations between FA and the expression intensity of RGMa protein was analyzed by Spearman correlation test.

Results As shown in Table 1, the FA value of bilateral cerebellar hemispheres at different time point after stroke onset was significantly decreased compared to the normal control group (P<0.05). The FA value reached the bottom at 12h and then climbed to a higher value than the first onset, but still signifantly lower than the control group (P<0.05). Compared to the ipsilateral (left) cerebellar hemisphere, the contralateral (right) cerebellar hemisphere revealed a slightly strong decline. As shown in Table 2, compared to the normal control group, the RGMa protein expression was significantly increased in the bilateral cerebellar hemispheres. The intensity of RGMa protein expression reached the peak at 24h after stroke onset. And compared to the ipsilateral (left) cerebellar hemisphere, the contralateral (right) cerebellar hemisphere showed a larger degree of increase. There was negative correlation between changes of FA values and the expression intensity of RGMa protein in the bilateral cerebellar hemispheres in MCAO group in this continuous time points (Spearman correlation test, the correlation coefficient r=-0.341,P<0.05).

Discussion and Conclusion In this study, FA value of DTI and the expression intensity of RGMa protein at the continuous time point were obtained and analyzed in remote regions of the infarct core in the rat MCAO model. This result confirmed that the MR DTI technology and the pathological examination of RGMa protein can reflect the occurrence of crossed cerebellar diaschisis after supratentorial infarction, and interprete the relevant mechanisms of CCD [2], which may provide a new, non-invasive technique to observe the secondary changes of the distant regions after supratentorial infarction.

Table 1. The FA values of the normal control group and MCAO group at different time point

Cerebellum		Control group	MCAO group						
			1h	3h	6h	9h	12h	24h	72h
FA	Right	0.298±0.031	0.245±0.041*	0.244±0.043*	0.237±0.064*	0.236±0.045*	0.228±0.014*	0.250±0.042*	0.265±0.024*
	Left	0.299±0.033	0.256±0.060*	0.253±0.052*	0.251±0.021*	0.246±0.049*	0.244±0.033*	0.265±0.033*	0.269±0.034*

* Two-sample paired t test, P<0.05

Table 2. The expression intensity of RGMa protein of the normal control group and MCAO group at different time point

Cerebellum		Control group	MCAO group						
			1h	3h	6h	9h	12h	24h	72h
FA	Right	84.03±2.93	101.50±4.42*	111.05±5.57*	113.80±7.56*	118.52±9.59*	120.23±7.68*	136.44±5.10*	122.96±9.80*
	Left	83.06±2.87	95.38±2.56*	100.98±4.32*	103.28±9.71*	103.71±6.73*	105.27±2.50*	120.74±5.06*	109.46±5.27*

* Two-sample paired t test, P<0.05

Reference

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[2] Schwab J, et al. Neurosci, 2005(21): 1569-1576