Spatial cognitive capability of rats with lateral temporal cortical infarction and intervention effect of thrombolytic capsule

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Received: 2005-05-04 **Accepted:** 2005-07-20 (14/XM)

Abstract

BACKGROUND: Temporal cortex is related to human and animal's learning and memory capability, it not only accepts the casting fibers of auditory sense, but also closely connects with many brain areas where are related to spatial cognition.

OBJECTIVE: To observe the improving effect of thrombolytic capsule on spatial cognitive capability of rats with lateral temporal cortical infarction

DESIGN: Completely randomized controlled study

SETTING: Department of Neurology, the 175th Hospital of Chinese PLA; Department of Neurology, General Hospital of Chinese PLA; Institute of Psychology of Chinese Academy of Science

MATERIALS: This experiment was carried out at Brain Function Laboratory of Institute of Psychology of Chinese Academy of Science from August 1998 to February 1999, 28 SD rats were randomly divided into 3 groups, namely brain stroke group with 10 rats, treatment group with 10 rats and sham operation group with 8 rats.

METHODS: Brain infarction model was established on SD rats by using three-dimensional directional photochemistry induction. Rats were anaesthetized with hydrochloride and intravenously injected of rose pink solution in dosage of 20 mg/kg from tail vein. Then incision was made on the left temporal scalp to expose temporal bone, cold photosource with the guidance of optical fiber was used for directional illumination for 20 minutes in area of 2 mm^2. Twenty minutes later, rats in treatment group were given intraperitoneal injection of thrombolytic capsule physiological saline of 40 g/L in dosage of 200 mg/kg, which replaced by the same volume of physiological saline in brain stroke group. Rats in sham-operation group received the same operative lamination and injected with the same volume of physiological saline from tail vein and intraperitoneal cavity.

MAIN OUTCOME MEASURES: Achievement of Morris water labyrinth training of all rats, including: ① Response time. ② Searching strategy (edge type scored 1, randomize type of 2, trend type of 3 and straight-line type of 4).

RESULTS: Data of 28 rats was entered the result analysis. ① The average response time: it was shortened in treatment group and sham operation group than brain infarction group [on 1st day: 58,50,65 s; 2nd day: 24,27, 46 s; 3rd day: 14,17,20 s; 4th day: 11,9,15 s, ($P < 0.01$)], but it was similar between treatment group and sham operation group ($P > 0.05$). ②Searching strategy grades: on the 1st day, scores were slightly higher in treatment group and sham operation group than infarction group, but were instable ($t=1.167$, $P > 0.05$). On the 2nd day, it was higher in sham operation group than brain stroke group[2.9,2.3, ($t=5.5$, $P < 0.05$)], but it was similar between treatment group and infarction group[2.6,2.3 scores, ($t=3.34$, $P > 0.05$)]. on the 3rd and 4th day, it was higher in treatment group than infarction group [3.5,2.7 minutes; 3.7,3.3 minutes, ($F=8.92$, 14, $P > 0.05$)] and similar between treatment group and sham operation group ($P > 0.05$).

CONCLUSION: Rat response time was reduced by thrombolytic capsule during the early time of cerebral infarction, changing searching tactics gradually from randomized and edge type to tendency type and straight line type, suggesting thrombolytic capsule can improve the post-ischemia cognitive function, raise training record of cerebral infarctional rats.

Dong MT, Guan XZ, Kuang PZ, Gao Y, Guan LC, Kuang PG, Qian HK.Spatial cognitive capability of rats with lateral temporal cortical infarction and intervention effect of thrombolytic capsule. *Zhongguo Linchuang Kangfu* 2005;9(32): 223-5(China) [www.zglckf.com]

INTRODUCTION

Temporal cortex is closely related to human and animal's learning and memory capability, it not only accepts the casting nuerofibers of hearing sense, but also closely connected with many subcortical regions where are related to spatial cognitive capability such as parietal frontal lobe, occipital lobe, midbrain, hippocampus and striatum. Pervious reports indicated that lateral temporal cortical infarction would result in spatial cognitive impairment in rats[1]. In this experiment, thrombolytic capsule (earthworm kinase preparation)was used in the treatment of lateral temporal cortical infarction and to observe the improvement of cognitive capability with the assistance of Morris water labyrinth test.

MATERIALS AND METHODS

Materials

This experiment was carried out at Brain Function Laboratory , Institute of Psychology of Chinese Academy of Science from August 1998 to February 1999, 28 healthy male SD rats, clearage grade, with body mass of (200±20)g, provided by the Experimental Animal Center of General Hospital of Chinese PLA, raised in room temperature, freely taking food and water during the experiment, were randomly divided into 3 groups, namely brain stroke group with 10 rats, treatment group with 10 rats and sham operation group with 8 rats, All tests and training should be carried out during 8: 30-17: 00. Thrombolytic capsule was purchased from Shanxi Yuanwei medicine limited company [the authorized document number (96) medical certification Z-32 and batch number: 790202], composed with 40 g/L physiological saline for future use.

Methods

Animal model preparation: brain infarction model was established on SD rats by using three-dimensional directional photochemistry induction. Rats were anaesthetized with hydrochloride (350 mg/kg) and fixed on stereo-orientor before slowly injected of rose pink solution in dosage of 20 mg/kg from tail vein within 3.0-4.0 minutes. Incisions were made vertically to cross the middle point between left temporal external ear path and outer canthus, muscles were separated to expose roots of zygoma and temporal bone, taking the midpoint of zygoma root as posterior line and root level as the lower limit, cold photosource of halogen lamp was guided by the optical fiber to directionally illuminate the exposed skull for 20 minutes, with intensity of 75 W and area of 2 mm^2. Rats in treatment group were given intraperitoneal injection of 40 g/L thrombolytic capsule physiological saline in dosage of 200 mg/kg, which replace by the same volume of physiological saline in brain stroke group. Rats in sham-operation group received the same operative illumination and injected with the same volume of physiological saline from tail vein and intraperitoneal cavity.

The Morris water labyrinth behavioral test installation and experimental process Morris water labyrinth is cream color opaque cylinder (diameter of 94 cm and height of 55 cm), equipped with transparent glass round platform (diameter of 6 cm), the top of which is 2 cm below the surface of cream color liquid. Rats in each group were another medication 48 hours after operation before starting

ISSN 1671-5926 CN 21-1470/R
www.zglckf.com kf23385083@sina.com

the first experiment; rats were subjected to Morris behavior experiment at 27 hours after operation. The experiment was divided into two stages, which was lasted altogether for 5 days including pretraining of 1 day and advanced training of 4 days, pretraining was not counted but the achievement of the following 4 days were recorded, rats were let to enter into the water from the edge of other three quadrants than the platform quadrant, the entering position was randomly selected. Rats were selected in turn within a group, 6 times /day a rat. Picture monitor system was used to trace their swimming path, and related data were recorded. Experimental parameters include: ① response time (s). ② Traveling course (not recorded because of breakdown). ③ Search strategy (edge type of 1 score, randomized type of 2 scores, tendency type of 3 scores and straight-line type of 4 scores).

Statistical analysis: SPSS 6.0 software package was used by the fourth author, the variance analysis of the experimental data was carried out for the training achievement comparison between groups.

RESULTS

Quantitative analysis of the experimental animals

Twenty-eight rats enrolled in this study and all complete the model preparation and training, the achievement of training was all recorded and entered result analysis.

Comparison of the average response time between groups

In the achievement of the 1st day training, there was not obviously different between groups, but from onset of the 2nd day, it was obviously shortened in the treatment group and sham operation group than cerebral infarction group (F=8.18, $P < 0.05$; F=9.83, $P < 0.01$) and the average response time of infarction group gradually adjacent to that of sham operation group along with the training days, but the difference on the 4th day was still significant difference (F=4.90, $P < 0.05$) (Table 1).

Table 1 Comparison of the average response time among groups (s)

Group	n	1st day	2nd day	3rd day	4th day
Cerebral infarction	10	65	46	20	15
Treatment	10	58	24	14	11
Sham operation	8	50	27	17	9

Search strategy of the rats in each group (Table 2)

Table 2 Search strategy of the rats in each group (scores)

Group	n	1st day	2nd day	3rd day	4th day
Cerebral infarction	10	2.1	2.3	2.7	3.3
Treatment	10	2.2	2.6	3.5	3.7
Sham operation	8	2.4	2.9	3.4	3.7

The search strategy can reflect the improvement stability of rat learning and memory, and the means of scores was used for comparison, on the 1st day, learning and memory was obviously improved but instable, so difference was of no statistical significance (F=1.167, $P > 0.05$), on the 2nd day, the difference between sham operation group and infarction group was remarkable (F=5.5, $P < 0.05$), moreover even remarkable on the 3rd and 4th day (F=8.92, F=14, $P < 0.01$), while there was no statistical difference between treatment group and sham operation group.

DISCUSSION

The photochemistry induced rat cerebral infarction model is a mature animal model for study of cerebra infarction inside and outside, especially in respect of thrombolytic treatment. Previous experiments demonstrated that lateral temporal cortical infarction would lead to rat spatial cognitive learning obstacle in the Morris water labyrinth[1], the results of present experiment is consistent with previous reported, suggesting that for its stability and higher repeatability, such experimental model is considered as an ideal animal model for the study of cerebral infarction. Temporal hippocampus is the main structure for involving in the spatial cognitive process with reference of dissidence of rat, but no the only spatial cognitive structure. "The chart cognition" theory was proposed aiming at the spatial cognition capability, believing that normal rat would adopt chart cognition processing way when carrying on spatial learning memory, and forming the cognition chart is the basic function of hippocampus. Meanwhile chart cognition process could be influenced by some other brain structures, presented by pure damages of frontal-parietal lobe, temporal lobe and caudal nucleus, septum could also result in spatial cognitive obstacle. The integrity of cognitive capability also depends on the fibre connecting integrity between subcortical brains regions [2,3].

Cerebral infarction model was established on rats by suing photochemistry induction, pathological thrombotic process was similar to that of human cerebral infarction, moreover, cognitive obstacle was also similar to vascular dementia. From the results we observed that response time of treatment group obviously reduced after thrombolytic capsule administration, and there was remarkably different from cerebral infarction group, but not obviously different from sham operation group. From respect of searching strategy, rats in all groups displayed transformation from edge type and random type to tendency type and straight-line type, which appeared late than responding time, but it keeps stable once established. Similar to response time, treatment group and sham operation group scored higher without statistical significance between them and cerebral infarction group scored lower and quite different from the former two groups. Some believed that cerebral infarction induced spatial cognitive obstacle was relative short, which was possibly due to the lateral infarction which could be compensated by the normal brain [4], along with the training times, difference in training achievement between cerebral infarction group and sham operation group was gradually reduced, but still showed statistical significance.

earthworm kinase is a proteinase extracted from earthworm [5,6]. From onset of 1980s, it has been studied and applied in clinical practice from 1990s, considered as a novel, economic and convenient thrombolytic preparation, its functions in dissolving thrombus, reducing platelet congregation and blood viscosity, as well as improving microcirculation and neural defect symptom of cerebral infarctional patient has been reported and began to be applied for treating other some thrombotic disease [7].

From the results of present experiment, earthworm kinase thrombolytic capsule was used in the treatment of cerebral infarction during acute period and proved to be capable of improving the cognition function, thereby considered effective in treating cerebral infarction and vascular dementia. But whether the improvement of post-infarction cognitive learning capability is correlated with its neurprotection function still needs further study[7].

REFERENCES

1 Sui N,Yang JJ,Kuang PZ. Effct of left temporal infarction of rats on spatial cognition. *Xinli Xuebao*(心理学报)1998;30(3):320-1
2 Sui N.Xie D,Kuang PZ. Effect of amygdalae lession on spatial cognitive. *Xinli Xuebao*(心理学报) 1995;27(3):311-2
3 Sui N,Weng XC,Gao Y, *et al.* Lesions of cpyr and globus pallidus impair mapping cognitive ability in the morris maze. *Xinli Xuebao*(心理学报)1995;27(4):428
4 Lyden PD, Zivin JA, Chabolla DR, *et al.* Quantitative effects of cerebral infarction on spatial learning in rats.*Exp Neurol* 1992;116(2):122-32
5 Nakajima N, Mihara H, Sumi H. Characterization of potent fibrinolytic enzymes in earthworm, Lumbricus rubellus. *Biosci Biotechnol Biochem* 1993;57(10):1726-30
6 Mihara H, Sumi H, Yoneta T, *et al.* A novel fibrinolytic enzyme extracted from the earthworm, Lumbricus rubellus.*Jpn J Physiol* 1991;41(3):461-72
7 Zhang GP,Qian RZ,Yang SC. Inhibitory assenble of platelet on lumbrukinase. am experimental observation. *Zhonghua Yixue Zazhi*(中华医学杂志)1998;79(6):394

单侧颞叶皮质脑梗死大鼠空间认知能力与溶栓胶囊的干预效应

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摘要
背景:颞叶皮质与人类和动物的学习记忆能力有关,它不仅接受听觉的投射纤维,还与许多空间认知相关的脑区有密切关系。
目的:观察单侧颞叶皮质脑梗死大鼠经溶栓胶囊治疗后空间认识能力的改善情况。
设计:完全随机对照实验。
单位:解放军第一七五医院神经内科,解放军总医院神经内科及中国科学院心理研究所。
材料:实验于1998-08/1999-02在中国科学院心理研究所脑功能研究室完成,选择SD大鼠28只,随机分为3组:脑梗死组10只,治疗组10只,假手术组8只。
方法:立体定向光化学诱导制作脑梗死大鼠模型。水合氯醛麻醉,尾静脉注射玫瑰红溶液20 mg/kg,左侧颞部切开头皮,暴露颞骨,光导纤维引导卤素灯冷光源,定向照射20 min,面积2 mm²。照射20 min后治疗组腹腔注射溶栓胶囊生理盐水溶液(40 g/L)200 mg/kg,脑梗死组腹腔内注射等量生理盐水。假手术组尾静脉及腹腔注射生理盐水外,手术照射条件相同。
主要观察指标:各组大鼠在Morris水迷宫中的训练成绩,包括:①反应时。②搜索策略(分为边缘式记1分,随机式记2分,趋向式记3分,直线式记4分)。
结果:28只大鼠均进入结果分析。①各组大鼠平均反应时比较:治疗组和假手术组短于脑梗死组[第1天:58,50,65 s;第2天:24,27,46 s;第3天:14,17,20 s;第4天:11,9,15 s,($P < 0.01$)],治疗组与假手术组基本接近($P > 0.05$)。②搜索策略评分:第1天可见假手术组及治疗组稍高于脑梗死组,但不稳定(F=1.167,$P > 0.05$),第2天假手术组高于脑梗死组[2.9,2.3分,(F=5.5,$P < 0.05$)],但治疗组与脑梗死组基本接近[2.6,2.3分,(F=3.34,$P > 0.05$)],第3,4天治疗组均高于脑梗死组[3.5,2.7分;3.7,3.3分,(F=8.92,14,$P < 0.01$)]。治疗组与假手术组基本接近($P > 0.05$)。
结论:脑梗死早期给予溶栓胶囊治疗后,反应时明显缩短,搜索策略逐渐由边缘式和随机式过渡到趋向式和直线式,说明溶栓胶囊可改善脑缺血后的认知功能障碍,提高脑梗死大鼠的训练成绩。
主题词:脑梗塞/药物疗法;胶囊;颞叶;空间知觉;迷宫学习
中图分类号:R749.1 文献标识码:A 文章编号:1671-5926(2005)32-0223-03
董闽田,管兴志,匡培梓,高扬,管林初,匡培根,钱惠康.单侧颞叶皮质脑梗死大鼠空间认知能力与溶栓胶囊的干预效应[J].中国临床康复,2005,9(32):223-5
[www.zglckf.com]
(Edited by Zhang FJ/ Sun SG / Song LP / Xiao XL)

·BASIC RESEARCH·

Expression of procaspase-3 in the mouse hippocampus after transient forebrain ischemia★

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Received: 2004-04-27 **Accepted:** 2005-05-11 (04/XN)

Abstract
BACKGROUND: After cerebral ischemia/reperfusion injury,as a executioner caspase, procaspase-3 and caspase-3-like activity increased significantly.We observe both the dephosphorylated and phosphorylated procaspase-3, and try to find out their variations during the processes of cerebral ischemia/reperfusion injury.
OBJECTIVE: Observe the expression of procaspase-3 in hippocampus following transient forebrain ischemia.
DESIGN: A randomized and controlled experiment.
DEPARTMENT: Department of Biochemistry and Molecular Biology, Capital University of Medical Sciences.
SETTING: Department of Hyperbaric Oxygen of Beijing Chaoyang Hospital Affiliated to Capital University of Medical Sciences.
METHODS: Transient forebrain ischemia was induced by bilateral common carotid occlusion (BCCAO) for 20 minutes.Hippocampus was obtained at reperfusion time points of 6 hours,12 hours,24 hours and 48 hours respec-tively after 20 minutes of BCCAO. Sham-operated group did not occlude bi-lateral common carotid,and hippocampus was obtained at reperfusion time point of 24 hours.Western Blotting was used to detect the level of procaspase-3.
MAIN OUTCOME MEASURES: The comparison of total procaspase-3, dephosphorylated procaspase-3 and phosphorylated procaspase-3 level in hippocampus between each group.
RESULTS: Thirty mice were involved in the analysis. ① Total procaspase-3 level: Total procaspase-3 level increased in hippocampus at reperfusion time points of 12 hours and 24 hours post-BCCAO (9 133.1±2 216.3, 9 355.9±1 901.6, $P < 0.05$). ②Dephosphorylated procaspase-3 level: The dephosphorylated procaspase-3 level increased in hippocampus at reperfusion time point of 24 hours post-BCCAO (7812.0±1625.1, 3825.8±155.6, $P < 0.05$). ③ Phosphorylated procaspase-3 level: The increased expression was not significant ($P > 0.05$) as compared with the expression levels in sham-operated mice.
CONCLUSION: Procaspase-3 is upregulated after ischemia/reperfusion. The increment of dephosphorylated form of procaspase-3 was higher than that of phosphorylated form of procaspase-3 upon cerebral ischemia/reperfusion injury, which indicates that cerebral ischemia/reperfusion injury possibly induced the dephosphorylation of procaspase-3 and promoted its transforming into activated form.

Liu TH,Chen R.Expression of procaspase-3 in the mouse hippocampus after transient forebrain ischemia.*Zhongguo Linchuang Kangfu* 2005;9(32):225-7(China)

INTRODUCTION
Cerebral ischemia/reperfusion injury will lead to neuronal apoptosis. Caspase-3 is an established protease known to be involved in neuronal apoptosis. Lots of evidences implicated that procaspase-3 and caspase-3-like activity increased significantly after cerebral ischemia/reperfusion injury[1-4]. Procaspase-3 has both dephosphorylated and phosphorylated form. There were a lot of evidences that the dephosphorylated form of procaspase-3 (32kDa) increased after cerebral ischemia/reperfusion, but little was known about the phosphorylated form of procaspase-3 (35kDa or so). In the present study, we examined both dephosphorylated and phosphorylated form of procaspase-3 after ischemia/reperfusion, and tried to find the possible mechanism of cerebral ischemia/reperfusion and the activation of procaspase-3.