中国临床康复 第9卷 第9期 2005-03-07 出版
Chinese Journal of Clinical Rehabilitation, March 7 2005 Vol. 9 No. 9

· BASIC RESEARCH ·

Effect of *angong niuhuang wan* on serum lactate dehydrogenase isoenzymes in rats with cerebral infarction*★

Tang Yi-shan, Pan Xin-hua, Wang Pei-xun, Wang Ning-sheng

Tang Yi-shan, Pan Xin-hua, Wang Pei-xun, Wang Ning-sheng, Institute of Clinical Pharmacology, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510405, Guangdong Province, China

Correspondence to: Tang Yi-shan★, Master, Associate investigator, Lecturer, Institute of Clinical Pharmacology, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510405, Guangdong Province, China
tangyishan@tom.com

Supported by: the National Climbing Plan Foundation of China(trying started project) *
Received: 2004-09-01 **Accepted:** 2004-11-01 (17/SX)

Abstract

BACKGROUND: *Angong niuhuang wan* consists of mercury and arsenic, which has drawn the attention on its safety. It is necessary to carry on the research on evaluation in its availability and safety.

OBJECTIVE: To study the mechanic differences of *angong niuhuang wan* in the organic body physiologically and pathologically.

DESIGN: Randomized controlled experiment based on the experimental animals.

SETTING: Institute of clinical pharmacology affiliated to a university.

MATERIALS: The experiment was performed in Institute of Clinical Pharmacology affiliated to Guangzhou University of Traditional Chinese Medicine from March to April in 2001. Guangdong Medical Experimental Animal Center provided 24 SD male rats, weighted varied from 250 g to 300 g.

METHODS: SD rats were randomized into 4 groups, named as normal group, normal & *angong niuhuang wan* group(normal & *wan* group), model of cerebral infarction group(model group) (The middle cerebral artery embolism was induced in rats by photochemistry.) and model of cerebral infarction group & *angong niuhuang wan* group (model & *wan* group), 6 rats in each group. Medication instruction: gastric feeding was applied once daily, 0.13 g/kg, totally for 7 days.

MAIN OUTCOME MEASURES: contents of lactate dehydrogenase (LDH) isoenzymes, LDH_{1-5}.

RESULTS: Contents of serum LDH_{1-3} in normal & *wan* group were significantly increased than those in normal group ($P < 0.01$), of which, the values of LDH_1, LDH_2, LDH_3 were(17.02±0.46)%, (14.70±0.18)%, (15.47±0.13)% successively in normal & *wan* group, were(11.25±0.70)%, (8.26±0.90)%, (12.86±0.90)% successively in normal group. Content of serum LDH_3[(15.51±2.60)% [in model & *wan* group was significantly increased compared with that in model group [(10.93±2.10)%] ($P < 0.01$). The contents of LDH_4 were[(22.62±3.00)%] and(28.18±0.80)% respectively in two groups, indicating significantly reducing($P < 0.01$).

CONCLUSION: The organic injury of *angong niuhuang wan* is less in pathological state of focal cerebral infarction than that in physiological state, suggesting that the mechanic differences of *angong niuhuang wan* in organic body present in physiological and pathological states.

Tang YS, Pan HX, Wang PX, Wang NS. Effect of *angong niuhuang wan* on serum lactate dehydrogenase isoenzymes in rats with cerebral infarction. *Zhongguo Linchuang Kangfu* 2005; 9(9): 242-3(China) [www.zglckf.com]

INTRODUCTION

Angong niuhuang wan(*san*) originates from *Treatise on Differentiation and Treatment of Epidemic Febrile Diseases* written by Wu Jutong in Qing dynasty, composed of 11 herbs, named as *niuhuang* (Calculus Bovis), *suiniujiao*(Pulvis Cornus Bubali) concentrated powder, *shexiang*(Moschus), *zhenzhu*(Margarita), *zhusha* (Cinnabaris), *xionghuang*(Realgar), *huanglian*(Rhizoma Coptidis), *huangqin* (Radix Scutellariae), *zhizi*(Fructus Gardeniae), *yujin*(Radix Curcumae), *bingpian* (Borneolum)[1], *etc.* It acts on clearing away heat, detoxification, resolving phlegm and opening orifice, and presents significant therapeutic effects on emergent treatments of severe cerebral injury. Since the main components of the formula are cinnabar(HgS) and realgar(As_4S_4), the safety of the formula is questioned, which has become the main obstacle in the export of patent Chinese herbal medicine. This experiment was to observe the mechanic differences of *angong niuhuang wan* in organic body in physiological and pathological states so as to provide evidences for its availability and safety.

MATERIALS AND METHODS

Materials

The experiment was accomplished in Institute of Clinical Pharmacology affiliated to Guangzhou University of Traditional Chinese Medicine from March to April in 2001, and the rat model of cerebral infarction was prepared in Oncoma Institute of Zhongshan University. Animals and group divisions: Totally 24 SD male rats, weighted varied from 250 g to 300 g, were employed, which were healthy and in common grade, provided by Guangdong Medical Experimental Animal Center(Qualified No. 2001A024). Four groups were randomized, named as normal group, normal & *angong niuhuang wan* group(normal & *wan* group), model of cerebral infarction group (model group) and model of cerebral infarction group & *angong niuhuang wan* group(model & *wan* group), 6 rats in each group.

Drugs and reagents: ① *angong niuhuang wan*(provided by First Guangdong Foshan Pharmaceutical Factory) ② Pentobarbital sodium 20 g/L. ③ Disodium-tetracholoride-fluorescein(rose Bengal B) 30 g/L. ④ Sodium carbozymethyl cellulose (CMC-Na) solution 3 g/L. ⑤ Reagents required for the assays of lactate dehydrogenase (LDH) isoenzymes with agarose gel electrophoresis method[2].

Main instruments: There were 360-argon-ion-laser(Nanjing Electron Tube Factory), craniotomies, agarose gel electrophoresis apparatus and UPV GDS7600 gel image-forming system.

Methods

Preparation for model of cerebral infarction in rats: Anesthesia was done with intra-abdominal injection with pentobarbital sodium 40 μg/g(mass weight). A slight arch incision was done perpendicularly from the midpoint between the eye and ear on the same side, about 1.5 cm. The temporal muscle was separated till to the conjunction with zygomatic bone and was cut at the posterior 1/2. The squamoparietal protuberance was exposed anterior and superior to the oval foramen. A bony window was made, 5 mm in diameter, by a dental driller at 2 mm anterior end to the conjunction between zygomatic arch and temporal squamotemporal bone. The middle cerebral artery was exposed by opening dura mater of brain. After injected rose Bengal B from femoral vein(25 mg/kg), the photoconductive beam from laser(560 nm in wave, 300 W in power) was radiated on middle cerebral artery for 10 minutes, and suture on the incision was followed[3].

Medication instruction: In normal & *wan* group and model & *wan* group, *angong niuhuang wan* was prescribed for gastric feeding once daily, 0.13 g/kg(mixed evenly with 3 g/K CMC-Na in a proper dosage), totally for 7 days. In normal and model groups, physiological saline of equal dosage was prescribed for gastric infusion.

Index assays: Blood was collected from the orbit after 7 days and the serum was separated(be careful to avoid hemolysis. Agarose gel

electrophoresis apparatus[2] was applied to determine LDH_{1-5}.

Statistical analysis: By adopting SPSS 10.0 software, the first writer managed data analysis and results with t test.

RESULTS

Quantitative analysis of the experimental animals

Totally 24 rats were included in the experiment, which was randomized into 4 groups, 6 rats in each. All the rats entered the analysis of the results.

Effect of *angong niuhuang wan* on serum LDH isoenzymes in rats with cerebral infarction (Table 1)

Table 1 Comparison of serum lactate dehydrogenase (LDH) isoenzymes in rats of every group ($\bar{x} \pm s$, $n = 6$, %)

Group	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Normal	11.25 ± 0.70	8.26 ± 0.90	12.86 ± 0.90	30.10 ± 1.20	37.53 ± 2.70
Model	13.30 ± 1.20[a]	10.13 ± 1.70[b]	10.93 ± 2.10	28.18 ± 0.80[a]	37.46 ± 3.20
Normal & *wan*	17.02 ± 0.46[a]	14.70 ± 0.18[a]	15.47 ± 0.13[a]	22.97 ± 0.56[a]	29.84 ± 0.41[a]
Model & *wan*	12.33 ± 0.70[a]	10.99 ± 1.10[a]	15.51 ± 2.60[c]	22.62 ± 3.00[ac]	38.55 ± 2.60

[a] $t = 3.61, 16.87, 2.67, 17.19, 4.71, 7.03, 3.26, 13.19, 5.67, 6.90$, $P < 0.01$, *vs* the normal group; [b] $t = 2.38$, $P < 0.05$, *vs* the normal group; [c] $t = 3.36, 4.39$, $P < 0.01$, *vs* the model group

DISCUSSION

LDH is one of the key enzymes of glycolysis and distributed in obvious histological specialty. LDH_1 and LDH_2 are mostly distributed in cardiac muscle, kidney and erythrocytes, LDH_3 mostly in spleen, pancreas, thyroid gland, adrenal gland and lymph node and LDH_4 and LDH_5 mostly in skeleton muscles and liver. When organic disorders happen, isoenzymes released in blood are various in percentages, therefore, the changes of serum LDH isoenzyme spectrum can reflect the location of sickness and severity of injury sensitively and precisely. For instance, LDH_1 and LDH_2 are increased significantly in cardiac embolism, viral or rheumatic myocarditis and Ke-shan disease; the general activity of LDH and LDH_5 increased remarkably during injury of hepatic cells and the activity of LDH_{2-4} increased during kidney damage[4-6].

In the experiment, serum LDH_{1-3} level in normal & *angong niuhuang wan* group was increased significantly compared with the control ($P < 0.01$), indicating that long-term taking *angong niuhuang wan* in normal physiological state can induce a certain of injury to cardiac muscle, kidney, erythrocytes and spleen. Only serum LDH_3 was increased ($P < 0.01$) and LDH_4 decreased significantly ($P < 0.01$) in model & *angong niuhuang wan* group compared with model group, suggesting that *angong niuhuang wan* causes less damage in the pathological state of focal cerebral infarction than that in normal physiological state. It is to infer that Cinnabaris and Realgar in angong niuhuang wan, manifest rather lower toxicity in proper pathological state, by probably coordination compound formed with some endogenous components. Wang *et al*[7] synthesizes in imitation arsenic-cysteine coordination compound, LD_{50}, 650 mg/kg, that is lower than AS_2O_3 LD_{50} (150 mg/kg), explaining the antagonism of cysteine to arsenic toxicity. In recent years, the researches of the biological effects on arsenide have deepened to molecule and gene and have discovered that micro-arsenic provides some positive reactions[8, 9], its organic toxicity intensity is not only related to its dosage and existed state, but also to antagonism or coordination with other substances and physiological and pathological states in individual.

To conclude, the injury of *angong niuhuang wan* in the pathological state of focal cerebral infarction is less than that in normal physiological state, suggesting the different effects of *angong niuhuang wan* on the organic body in physiological and pathological states.

REFERENCES

1 National pharmacopoeia committee. *Pharmacopoeia of People's Republic of China*. 2000 edition. Beijing: Chemical Industry Publishing House 2000: 460
2 Wang ZF, Sun XH, Du J, *et al*. Improvement of agarose gel electrophoresis method on testing isoenzymes of lactate dehydrogenase. *Zhonghua Yixue Jianyan Zazhi* (中华医学检验杂志) 1992; 15(5): 315
3 Wang W, Dong WW, Fu YG, *et al*. middle cerebral artery occlusion and recanalization of rats by photochemical induction. *Zhongguo Shenjing Jingshen Jibing Zazhi* (中国神经精神疾病杂志) 1996; 22(1): 27－30
4 Li E, Wang SC. *Lecture Election of Foundation and Clinics* (the last volume). Beijing: People's Medicine Publishing House 1982: 11
5 Zhou ZJ, Zeng CZ. Clinical significance of serum lactate dehydrogenase and creatine phosphokinase in brain infarct patients. *Shenjing Jibing yu Jingshen Weisheng* (神经疾病与精神卫生) 2003; 3(4): 292－3
6 Qiu XH, Liu JY, Qing J. Effects of peroxidized lipids, enzymes and trace elements in serum in the generation of cerebral infarction. *Zhongguo Linchuang Kangfu* (中国临床康复) 2003; 7(16): 2378－9
7 Wang XP, Cao ZQ, Lu XY. The study of state of the heavy metal in the Liu Shen Pill and interact with arganic constitnents. *Weiliang Yuansu yu Jiankang Yanjiu* (微量元素与健康研究) 2003; 20(4): 27－8
8 Pan ZM, Liu KT, Yang L, *et al*. Progress of studies on effects of arsenic on cells and organisms. *Huanjing Jiankang Zazhi* (环境健康杂志) 2003; 20(3): 184－6
9 Pan ZM, Yang L, Liu KT, *et al*. Effects of arsenic compounds on human metallothionein-3 cDNA gene expression. *Huanjing Jiankang Zazhi* (环境健康杂志) 2004; 21(4): 221－3

安宫牛黄丸对脑梗死大鼠血清乳酸脱氢酶同工酶的影响**★

汤毅珊,潘华新,王培训,王宁生(广州中医药大学临床药理研究所,广东省广州市 510405)
汤毅珊★,女,1970年生,广东省广州市人,汉族,1996年华南理工大学毕业,硕士,助理研究员,讲师,主要从事中药有效性与安全性评价和中药药代动力学研究。
国家攀登计划资助项目(试启动课题)*

摘要

背景:安宫牛黄丸因含有汞、砷成分,其安全性受到关注,有必要进行有效性和安全性评价研究。
目的:研究生理、病理状态下安宫牛黄丸对机体作用的差异。
设计:以实验动物为研究对象的随机对照实验。
单位:一所大学的临床药理研究所。
材料:实验于2001－03/04在广州中医药大学临床药理研究所完成,广东省医学实验动物中心提供体质量250～300 g SD雄性大鼠24只。
方法:SD大鼠随机分成4组:正常组;正常＋安宫牛黄丸组;脑梗死模型组(光化学诱导大鼠大脑中动脉闭塞);脑梗死模型＋安宫牛黄丸组。每组6只。给药方法:每天胃饲1次,0.13 g/kg,共7 d。
主要观察指标:血清乳酸脱氢酶同工酶LDH_{1-5}含量。
结果:正常＋安宫牛黄丸组大鼠血清LDH_{1-3}含量比正常组显著升高($P < 0.01$),其中正常＋安宫牛黄丸组LDH_1,LDH_2,LDH_3的值分别为(17.02 ± 0.46),(14.70 ± 0.18),(15.47 ± 0.13)%,正常组则为(11.25 ± 0.70),(8.26 ± 0.90),(12.86 ± 0.90)%;模型＋安宫牛黄丸组大鼠血清LDH_3(15.51 ± 2.60,%)比模型组(10.93 ± 2.10,%)显著升高($P < 0.01$),LDH_4含量显著降低,其值分别为(22.62 ± 3.00)%,(28.18 ± 0.80)%($P < 0.01$)。
结论:在局灶性脑梗死病理状态下安宫牛黄丸对机体的损伤作用比在正常生理状态下小,提示安宫牛黄丸在生理、病理状态下,对机体的作用方式存在差异。

主题词:安宫牛黄丸;乳酸脱氢酶同工酶类;脑梗死
中图分类号:R743 **文献标识码**:A **文章编号**:1671－5926(2005)09－0242－02
汤毅珊,潘华新,王培训,王宁生. 安宫牛黄丸对脑梗死大鼠血清乳酸脱氢酶同工酶的影响[J]. 中国临床康复,2005,9(9):242－3 [www. zglckf. com]
(Edited by Wang F/Ji H/Wang L)