dose-depended manner. Moreover, morphological observation also proves that morphological change of PC12 cells under hypoxia state can be markedly improved by *xingnao qizhi* drug, which is presented by better refraction and decreased cell fragments. Thus, it can be concluded that *xingnao qizhi* capsule can attenuate cell hypoxia injury, and it might be one of the pharmacological mechanisms for the prevention and treatment of IR-induced learning memory disturbance.

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醒脑启智胶囊药物血清对PC12细胞缺氧损伤的保护作用*

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摘要

背景:血管性痴呆的发生与脑缺血缺氧河北省有关,前期的研究表明临床效方—醒脑启智胶囊可明显改善血管性痴呆小鼠的行为学障碍,但其作用途径是否为保护缺氧细胞从而改善血管性痴呆症状。

目的:采用血清药理学方法,体外培养嗜铬细胞瘤PC12细胞,用$Na_2S_2O_4$产生缺氧损伤,观察醒脑启智药物血清是否对缺氧损伤的PC12细胞具有保护作用。

设计:随机对照实验。

单位:河北医科大学中医学院。

对象:实验于2003-06/12在河北医科大学动物实验室和细胞培养实验室进行。40只SD大鼠随机分成5组,即对照血清组(n=12)和醒脑启智药物血清25.42,12.71,6.35,3.18 g/kg组(n=7)。PC12细胞株购自中国医学科学院细胞中心。

方法:①药物血清制备:醒脑启智药物血清25.42,12.71,6.35,3.18 g/kg组按相应剂量以醒脑启智药物(枸杞子、石菖蒲、川芎、橘络等)灌胃给药,对照血清组以生理盐水10 mL/kg灌胃,均为2次/d,每天灌胃间隔12 h,连续给药3 d,末次给药后1h麻醉状态下股动脉采血,分离血清。②PC12细胞分组培养:将体外培养的PC12细胞分为6组:对照组加入对照血清培养;模型组加对照血清培养1 h后加入$Na_2S_2O_4$产生缺氧损伤;醒脑启智25.42,12.71,6.35,3.18 g/kg组分别加入5%不相应剂量的药物血清,培养1 h后加入$Na_2S_2O_4$。③观察指标:各组细胞加入$Na_2S_2O_4$培养16 h后,倒置相差显微镜下观察PC12细胞形态变化;计算乳酸脱氢酶释放抑制率和PC12细胞损伤的抑制率(MTT法)来评估细胞活力。

主要结局观察:①各组PC12细胞的形态学观察。②各组PC12细胞的乳酸脱氢酶活性释放抑制率和PC12细胞损伤的抑制率。

结果:①细胞形态:醒脑启智各剂量组PC12细胞折光性较好,细胞碎片形成减少。②乳酸脱氢酶活性释放抑制率:醒脑启智25.42,12.71,6.35,3.18g/kg组分别为81.6%,69.6%,54.4%,27.8%。③PC12细胞损伤的抑制率:醒脑启智25.42,12.71,6.35,3.18 g/kg组分别为82.9%,75.6%,65.9%,53.7%。

结论:醒脑启智药物血清能明显减轻PC12细胞的缺氧损伤,可增强细胞活力,降低乳酸脱氢酶活性,并呈现一定的剂量依赖关系。

主题词:痴呆,血管性/中药疗法;PC12细胞;缺氧
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·*BASIC RESEARCH*·

Effects of drug serum in broken *bushen yizhi* formulas on cell model of Alzheimer disease*★

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Abstract

BACKGROUND: Chinese herb, *bushen yizhi* formula protects at certain extent learning and memory in rat model of Alzheimer disease. The drug serum in this formula can alleviate neurotoxic reaction of nerve tumor cell NG 108-15 to beta-amyloid protein. In order to understand further the mechanism and compatibility of the formula, it is necessary to carry on the study on the broken formulas.

OBJECTIVE: To study the effect of drug serum in subgroups of broken *bushen yizhi* formulas on growth and differentiation of cell model of Alzheimer disease and probe into the compatibility rule of *bushen yizhi* formula in view of serum pharmacology.

DESIGN: Randomized controlled experiment.

SETTING: DME Center of Clinical Pharmacological Institute Affiliated to Guangzhou University of Traditional Chinese Medicine.

PARTICIPANTS: The experiment was performed in DME Center of Clinical Pharmacological Institute Affiliated to Guangzhou University of Traditional Chinese Medicine from January to August 2003, in which, 40 healthy male SD rats of 3 months old were employed and NG108-15 cell line was frozen-preserved.

METHODS: ①Preparation of drug serum: Forty SD rats were randomized into the control, original formula group (No.1 group) [*shechuanzi* (Cnidium monnieri (L.) Cuss), *gouqizi* (Lycium barbarum L.), *renshen* (Panax ginseng C.A.Mey.), *heshouwu* (Polygonum multiflorum Thunb.), *danpi* (Paeonia Suffruticisa Andr.) and *bingpian* (Borneolum)], kidney replenishment group (No. 2 group) [*shechuanzi* (Cnidium monnieri (L.) Cuss), *gouqizi* (Lycium barbarum L.), *etc*.], group for benefiting *qi* and nourishing blood (No.3 group) [*renshen* (Panaz ginseng C.A.Mey), *zhishouwu* (Polygonum multiflorum Thunb.), *etc*.] and group with *bingpian* (Borneolum) removed (No.4 group) [Panax ginseng C.A.Mey.], *heshouwu* (Polygonum multiflorum Thunb.) and *danpi* (Paeonia Suffruticisa Andr.)], 8 rats in each group. The concentrated Chinese herbal solutions of every group were applied at 10 μL/g (equal to 6 g/kg of raw herbs) for gastric infusion successively,continuously for 1 month. In the control, the physical saline solution of equal dosage was used for infusion. Two hours after the last gastric infusion in rats of each group, the blood was collected from heart after anesthesia and the serum was separated for preparation. ②Cell proliferation, culture and differentiation: NG108-15 cell cultured *in vitro* was divided into 6 groups. In the control and model group, normal rat serum was contained in proliferated culture solution. In the rest 4 groups, the drug serum of No.1 group and 3 sub-groups was contained.Simultaneously, beta-amyloid protein 25-35 in each hole was prepared to the terminal concentration 5 μmol/L (except in the control) and the culture went on for 48 hours.

MAIN OUTCOME MEASURES:MTT method was used to determine proliferated number and survival rate of cells. Simultaneously, the ratio of

neurite cells to total cell count and average length of neurit were determined.

RESULTS: ① Cell proliferation: A value in model group was lower significantly than the control (0.520±0.022, 0.665±0.037, $P < 0.01$), and that in every drug serum group was higher than model group, of which, the result in No. 4 group was the most significant (0.636±0.035, $P < 0.01$). ② Survival rate of differentiated cells: That in model group was lower significantly than the control (58.4%, 100%) and that in every drug serum group was higher than model group, of which, the result in No.4 group was the most significant (75.8%, $P < 0.01$). ③ Ratio of differentiated neurite cells to total cell count: That in model group was lower significantly than the control [(42.95±11.42)%, (58.75±12.84)%, $P < 0.01$] and that in every drug serum group was higher than model group, of which, the result in No.4 group was the most significant [(58.20±8.40)%, $P < 0.01$]. ④ Average length of neurite: That in model group was shorter significantly than the control [(356.0 ±109.0), (493.8±133.0) μm, $P < 0.01$] and that in every drug serum group was longer than model group, of which, the result in No.4 group was the most significant [(486.8±79.2) μm, $P < 0.01$].

CONCLUSION: The drug serum in all of *bushen yizhi* formula and every subgroup inhibits at certain extent the injury of beta-amyloid protein 25-35 to NG108-15 cell, but the results of each group are various. The protection of drug serum to the cell in every group is in the sequence from strong to weak as group with *bingpian* removed > original formula group > kidney replenishment group > group for benefiting *qi* and nourishing blood. It is to expect a further study on the efficacy of group with *bingpian* removed.

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INTRODUCTION

It is indicated in study over the past that *bushen yizhi* formula protects at certain extent the learning and memory in Alzheimer disease model[1].Drug serum in this formula increases the survival rate of NG108-15 cell injured by beta-amyloid protein 25-35 and cell neurite and reduces neurotoxic reaction of cell to beta-amyloid protein [2].In order to understand further the mechanism and com-patibility of the formula on Alzheimer disease,the writers broke the formula according to different efficacies based on theories of Chinese medicine and in experiment *in vitro*, serum pharmacologic method was used to observe initially the effects of drug serum in subgroups of broken formulas in model of Alzheimer disease.

MATERIALS AND METHODS

Materials

The experiment was performed in DME Center of Clinical Pharmacological Institute Affiliated to Guangzhou University of Traditional Chinese Medicine from January to August 2003,in which,40 healthy male SD rats of 3 months old were employed (clean grade, Ao No. 2000A019), mass weighted (200±20) g and provided from Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine. The rats were bred in separated cages, with natural lights, free of drinking and eating.

Cells:NG108-15 cell line was provided from Neural Biological Department of Japanese Kanazawa University.

Reagents:DMEM culture medium, fetus bovine serum (FBS),hypoxanthined/Thymidine (H/T) and aminopterin (A) from Gibco company,beta-amyloid protein 25-35 segment,cAMP bought from Sigma company; MTT (imported separated package) and DMF (imported separated package)bought from Shanghai Biologic Engineering Company and the rest reagents were from domestic analytically pure (AP).

Medicines: *bushen yizhi* formula (No.1 group)is composed of *shechuanzi* (Cnidium monnieri (L.) Cuss),*gouqizi* (Lycium barbarum L.),*renshen* (Panax ginseng C.A.Mey.), *heshouwu* (Polygonum multiflorum Thunb.), *danpi* (Paeonia Suffruticisa Andr.) and *bingpian* (Borneolum) at certain percentages and provided from Medicament Department of Guangdong Hospital of Chinese medicine.According to different efficacies, 3 subgroups were divided, named kidney replenishment group (No.2 group) [*shechuanzi* (Cnidium monnieri (L.) Cuss), *gouqizi* (Lycium barbarum L.), *etc.*], group for benefiting *qi* and nourishing blood (No.3. group) [*renshen* (Panaz ginseng C.A. Mey), *zhishouwu* (Polygonum multiflorum Thunb.), *etc.*] and group with *bingpian* (Borneolum) removed (No.4 group) [No.2 + No.3 groups]. The concentrated solutions of *bushen yizhi* formula and every subgroup were prepared in Preparation Room of Guangdong Hospital of Chinese Medicine. The main procedure of preparation was as follows: boiled water of proper amount was added in danpi (Paeonia Suffruticisa Andr) for extraction for 10 minutes and the filtered solution was cold preserved.*Renshen* (Panax ginseng C.A. Mey.) was cut into slice pieces and was water extracted twice. The two filtered solutions were mixed, concentrated and cold preserved. The residue of *danpi* (Paeonia Suffruticisa Andr) with boiled water extracted for 10 minutes was mixed with *shechuanzi* (Cnidium monnieri (L.) Cuss), *gouqizi* (Lycium barbarum L.) and *heshouwu* (Polygonum multiflorum Thunb.) and water extracted for three times. The filtered solutions were mixed together, concentrated and cold preserved for 24 hours, which were also mixed together with *renshen* (Panax ginseng C.A.Mey) extractive solution. Simultaneously, *bingpian* (Borneolum) dissolved with little amount of ethanol and *danpi* extractive solution were added and mixed together. Finally, distilled water was used to concentrate the extractive solution as 0.6 g/mL of raw herbs and cold preserved at 4 °C.

Methods

Preparation of drug serum

SD rats were randomized into the control, No.1 group (original formula group), No.2 group, No.3 group and No.4 group, 8 rats in each. Chinese herbal concentrated solutions of various groups were changed into rat dosage according to adult treatment dosage for gastric infusion, 10 μL/g each time (equal to 6 g/kg of raw herbs), continuously for 1 month. In the control, physical saline of equal dosage was used for infusion. Two hours after the last gastric infusion, after anesthesia with 200 g/L urethane, the blood was collected from heart and the serum was separated with centrifugation at 1 500 r/minute for 20 minutes at 4 °C. The serum of 8 rats in each group was mixed and inactivated for 30 minutes at 56 °C. After bacteria removal and filtration, the serum was preserved in refrigerator at 4 °C.

Culture of cell proliferation

According to the experimental results reported in the research group [2], the drug serum of 0.05 volume fraction was selected for cell culture. After unfrozen, NG 108-15 cell preserved in liquid nitrogen storage jar was centrifugated and the upper clear was removed. After rinsed with cell proliferated solution (DMEM culture solution with FBS of 0.05 volume fraction, and 10 g/L HAT, meaning H/T together with A), the cell was placed separately in cell culture bottle (NUNC) of 25 cm^2 and cultured in culture box with CO_2 of 0.1 volume fraction at 37 °C. After basically filled up, cell proliferated solution was used to dilute cell count up to 3×10^7 L^{-1}, and was generated to 96-hole culture plate (NUNC),100 μL in each hole. Normal rat serum was contained in proliferated solution in the holes in the control and model group and drug serum was contained in the rest 4 groups, named original formula group and 3 subgroups. Simultaneously, beta-amyloid protein 25-35 in each hole was prepared to the terminal concentration 5 μmol/L (except in the control) and the culture went on for 48 hours.

Culture of cell differentiation

Cell differentiating solution (DMEM culture solution contained FBS of 0.05 volume fraction, 10 g/L H/T, 1 mmol cAMP) was used to dilute NG 108-15 cell that was filled up in cell culture bottle of 25

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cm^2 up to $3\times10^7L^{-1}$. At 100 μL each hole, the cells were generated into 96-hole culture plate.The solution was replaced in each group with culture solution contained rat serum of 0.05 volume fraction (it was same as the above in subgroups). To be same, beta-amyloid protein 25–35 in each hole was prepared to the terminal concentration 5 μmol/L (except in the control) and the culture went on for 48 hours.

Observation and indexes and methods

Observation of cell morphology: DM reversed microscope from Leica company was used to observe the cells in 96-hole culture plate and pictured everyday.

Determination of cell proliferated counts and survival rate:MTT method[3] was used to determine the cells that had been proliferated or differentiated for 48 hours with rat serum and received corresponding absorbency *(A)* values of proliferating number and survival rate of the above two categories of cells.100 μL culture solution was removed from each hole and replaced with DMEM culture solution without serum contained. Simultaneously, 20 μL MTT was added, 4 hours later, mixed solution with 100 μL SDS of 200 g/L mass concentration and DMF of 0.5 volume fraction was added, which was over the night at 37 °C. On the second day, DG 3022 type enzyme linked immunoassay instrument (from Huadong Electronic Tube Factory) was used to determine *A* value at wave length of 570 nm.

Determination of ratio of differentiated neurite cells to total cell count and average length of neurite: Microscopic photography was done on cells after differentiation for 48 hours with rat serum. Four holes were randomized from each group and a picture was taken from upper, lower, left and right of each hole successively (the view basically covered entire hole). After film developed, DMR imaging analyzer from Leica Company was used to analyze each picture to receive the ratio of differentiated neurite cells to total cell count and average length of neurite.

Statistical analysis

The first writer used SPSS 10.0 software for analysis of variance on data in every group. The data in every group were expressed with Mean ± SD and significant level α=0.05.

RESULTS

Descriptive statistics

Chinese herbal gastric infusion and observation of drug serum preparation

During gastric infusion with Chinese herbs and normal breeding, no rat was died in each group. One month later, insoluble blood with drug serum was obtained.

Morphologic observation of cells in each group

NG 108-15 cell is neural tumor cell,which is formed by hybridism of cells of neural embryo blastoma in mouse and neuroglial cell tumor in rat. It can reproduce successively and infinitely, with neuronal neurite. In culture solution with or without cAMP differential agent, proliferating phase and differentiating phase present respectively[4]. 48 hours after serum action in every group, cell neurite in pro-liferating phase was shorter than differentiating phase,but cell count was more than that in differentiating state. It was indicated initially in observation of cell morphology in proliferating phase that cell amount in model group was decreased remarkably and a part of cells turned to be black, aggregated and died. In drug serum groups, a certain protection was obtained on neurons cultured *in vitro* that were injured by beta-amyloid protein with various degrees, of which, the result in No.4 group was better. It was displayed in observation of cell with differentiating culture that compared with the control, more cells turned to be black and died in every group with beta-amyloid protein; but in every drug serum group, there was still some long axons existed, which was especially more in compound group and No.4 group.

Statistical inference

Comparison of proliferated cell count and survival rate of differentiated cells (Table 1)

Table 1 Effects of drug serum in broken *bushen yizhi* formulas on NG 108-15 cell proliferation and survival rate of differentiated cells ($\bar{x}\pm s$, n=12)

Group	Survival rate of differentiated cells		
	Cell proliferation (*A*)	Absorbency	Survival rate (%)
Control	0.665±0.037	0.380±0.037	100.0
Model	0.520±0.022[a]	0.222±0.025[a]	58.4
Original formula	0.568±0.057[acd]	0.275±0.032[ac]	72.4
Kidney replenishment	0.558±0.042[abd]	0.260±0.025[ac]	68.4
Benefiting *qi* and nourishing blood	0.560±0.054[abd]	0.268±0.022[ac]	70.5
Borneolum removed	0.636±0.035[c]	0.288±0.044[ac]	75.8
F	19.38	33.25	
P	< 0.01	< 0.01	

[a]P < 0.01,*vs* control group;[b]P < 0.05,[c]P < 0.01,*vs* model group,[d]P < 0.01,*vs* Borneolum removed group

Comparison of percentage of differentiated neurite cells and average length of neurite in each group (Table 2)

Table 2 Comparison of percentage of differentiated synaptic cells and average length of neurite in each group ($\bar{x}\pm s$, n=12)

Group	Neurite rate (%)	Average length of neurite (μm)
Control	58.75±12.84	493.8±133.0
Model	42.95±11.42[b]	356.0±109.0[a]
Original formula	50.00±10.00[ace]	488.3±65.8[d]
Kidney replenishment	45.58±14.33[bf]	461.7±46.7[d]
Benefiting *qi* and nourishing blood	45.40±7.70[bf]	466.6±74.4[c]
Borneolum removed	58.20±8.40[d]	486.8±79.2[d]
F	7.32	6.77
P	< 0.01	< 0.01

[a]P < 0.05,[b]P < 0.01,*vs* control group;[c]P < 0.05,[d]P < 0.01,*vs* model group; [e]P < 0.05, [f]P < 0.01, *vs* Borneolum removed group

DISCUSSION

Bishen yizhi formula is designed for senile dementia according to etiology and pathogenesis in Chinese medicine, mainly composed of *shechuanzi* (Cnidium monnieri (L.) Cuss), *gouqizi* (Lycium barbarum L.), *renshen* (Panax ginseng C.A.Mey.), *heshouwu* (Poly-gonum multiflorum Thunb.), *danpi* (Paeonia Suffruticisa Andr.) and *bingpian* (Borneolum). In the formula, *shechuanzi* and *gouqizi* act on tonifying kidney *yin* and *yang*, benefiting marrow and filling up essence. *Renshen* and *heshouwu* act on benefiting *qi* and nourishing blood. *Danpi* acts on cooling blood and activating blood activity. *Bingpian* acts on opening orifices and retaining consciousness, which is taken as conductant herb, directing the actions of other herbs to the affected area so as to open the orifice. Based on the etiology and pathogenesis, the formula treats the symptoms and root cause of dementia simultaneously. In this experiment, due to the various efficacies of the formula, named tonifying kidney, ben-efiting *qi*, nourishing blood and opening orifice, the original formula (No.1 group) was broken into three subgroups, named group for kidney replenishment (No.2 group), group for benefiting *qi* and nourishing blood (No.3 group) and group with *bingpian* removed (No.4 group). It was to observe the possible protection of drug serum in every group on NG 108-15 cell injured by beta-amyloid protein 25–35 so as to probe into the regulation of compatibility of the formula. Beta-amyloid protein is the main component of senile plague in cerebral tissue in Alzheimer disease,its active region is on 11 amino acid segment from No.25 to No.35.When the diluted solution of beta-amyloid protein 25–35 is placed in culture box at 37 °C for"aging", toxic clusters will be produced[5].Before this experiment, the writers have observed the proliferating rate of NG 108-15 cell cultured in rat serum with different concentrations (0.05, 0.1 and 0.2 volume fractions). By further observation of the effects of beta-amyloid protein on the cell in various aging days (1, 4, 7 and 10 days), at various concentrations (5, 10, 20 μmol/L) and in various

durations of action (24, 48 and 72 hours), it is to choose that the most proper conditions for observation of drug serum action are in DMEM culture solution with rat serum of 0.05 volume fraction, beta-amyloid protein 5 μmon/L, aging of 4 days and for 48 hours. In this experiment, with 5 μmol/L beta-amyloid protein 25–35 for 48 hours, NG 108-15 cell morphological structure and growth and differentiation changed obviously compared with normal cultured cells. No matter in proliferating phase or differentiating phase, cell amount was decreased remarkably and a part of cells turned to be black, aggregated and died; the ratio of differentiated neurite cells to total cell count was reduced and average length of neurite was decreased remarkably, too. It is indicated that "aged" beta-amyloid protein 25–35 segment injures NG 108-15 cell cultured *in vitro*, inhibits growth and differentiation of cell and further induce cell death. Such cell injury model can imitate at certain extent the cerebral neuronal injury in Alzheimer disease.

It was indicated in the results of this experiment that compared with the state in cell model of Alzheimer disease, the drug serum in both original formula and every subgroup inhibited the injury of beta-amyloid protein 25–35 to the cell, but the results were various in different groups.At proliferating phase of NG 108-15 cell, the cell amount in No.4 group was more remarkably than the rest drug serum groups, which was basically same as normal control. At differentiating phase of NG 108-15 cell, due to the stop of cell proliferation and injury from beta-amyloid protein 25–35,cell amount in every drug serum group was decreased at different degrees and the survival rates of those were basically same, but still higher than model group.The differences among various drug serum groups were observed by further analysis on neurite rate of differ-entiated cell and average length of neurite.Compared with model group,the cell neurite rates in No.1 and No.4 groups were improved remarkably. In comparison among drug serum groups, the neurite rate in No.4 group was increased remarkably than the rest 3 groups. That in No.1 group was slightly increased compared with No.2 and No.3 groups. Concerning to average length of neurite in every drug serum group, the average length in 4 drug serum groups was longer than model group, close to the control. Simultaneously, that in No.4 group and No.1 group was all slightly longer than No.2 and No.3 groups. It was displayed in the pictures of cultured cell in every drug serum group that the cell growth in No.4 group was the best, that in No.1 and No. 2 were the second and that in No.3 group was the worst. In summary, the protection of drug serum to the cell in every group is in the sequence as No.4 group > No.1 group > No.2 group > No.3 group.

The action of No.1 and No.4 groups is superior to No.2 and No.3 group, indicating the coordination of the herbs in No.2 and No.3 groups. *It is verified initially in the experiment in vitro that the* compatibility of this formula is rational. No.2 group is superior to No.3 group, explaining that compared with No.3 group, the effec-tive components in drug serum of such subgroup probably plays the dominant role in protection of NG 108-15 cell injured by be-ta-amyloid protein 25–35. In this experiment, it is discovered that the action of No.4 group is stronger than No.2 group.It is indicated in recent study that bingpian promotes drugs penetrating blood-brain barrier[6]. Concerning to the treatment and clinical medication with *bushen yizhi* formula on holistic animal model of Alzheimer disease, *bingpian* protects cerebral neurons due to its promoting actions on effective components of drugs. It is to infer that in this experiment, *bingpian* included in *bushen yizhi* formula probably promotes the effective components in drugs penetrating blood-brain barrier and decreasing the concentrations in blood, resulting in the different effects of No.4 group and No.1 group on serum protection of NG 108-15 cell injured by beta-amyloid protein 25–35. Neverthe less,a further experiment *in vitro* (such as influence of cerebrospinal fluid of infused animal on cultured cell *in vitro*) is expected for verification.

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补肾益智方拆方含药血清对阿尔茨海默病细胞模型的作用 *★

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摘要

背景:中药补肾益智方对阿尔茨海默病大鼠模型学习记忆能力有一定的保护作用,该方含药血清也能减轻神经瘤细胞 NG 108-15 细胞对 β 淀粉样蛋白的神经毒性反应,为了进一步了解该方的作用机制及其配伍规律,需对该方进行拆方研究。

目的:研究补肾益智方拆方亚组的含药血清对阿尔茨海默病细胞模型生长、分化等方面的影响,从血清药理学的角度探讨该方的配伍规律。

设计:随机对照实验。

单位:广州中医药大学临床药理研究所 DME 中心。

对象:实验于 2003-01/08 在广州中医药大学临床药理研究所 DME 中心实验室进行。实验对象为 3 月龄 SD 雄性健康大鼠 40 只和冻存的 NG 108-15 细胞株。

方法:①制备药物血清:将 40 只 SD 大鼠随机分为对照组、原方组(蛇床子、枸杞子、人参、何首乌、丹皮、冰片)、补肾组(蛇床子、枸杞等)、益气养血组(人参、制首乌等)、去冰片组(蛇床子、枸杞子、人参、何首乌、丹皮),每组 8 只。将各组中药浓缩液按按 10 μL/g (相当于生药 6 g/kg)分别进行灌胃,连续 1 个月,对照组灌等量生量盐水。各组大鼠最后 1 次灌胃 2h,麻醉后心脏采血,分离血清备用。②细胞增殖、培养及分化:将体外培养的 NG 108-15 细胞分为 6 组,对照组、模型组孔中的增殖培养液含正常大鼠血清,其余 4 组分别为原方组及 3 个亚组的大鼠含药血清;同时每个孔加淀粉样 β 蛋白 25~35 至终浓度 5 umol/L(对照组除外),继续培养 48 h。

主要观察指标:用 MTT 法测定细胞增殖数和存活率;同时检测分化突起细胞占总细胞数的比率及突起平均长度。

结果:①细胞增殖情况: 模型组 *A* 值显著低于对照组 (0.520±0.022, 0.665±0.037,$P < 0.01$),各含药血清组 A 值均高于模型组,但以去冰片组效果最显著(0.636±0.035,$P < 0.01$)。②分化细胞存活率:模型组显著低于对照组(58.4%,100%),各含药血清组均高于模型组,但以去冰片组效果最显著(75.8%,$P < 0.01$)。③分化突起细胞占总细胞数的比率:模型组显著低于对照组[(42.95±11.42)%,(58.75±12.84)%,$P < 0.01$],各含药血清组均高于模型组,但以去冰片组效果最显著[(58.20±8.40)%,$P < 0.01$]。④突起平均长度: 模型组显著低于对照组 [(356.0±109.0),(493.8±133.0)μm,$P < 0.01$],各含药血清组均高于模型组,但以去冰片组效果最显著[(486.8±79.2) μm,$P < 0.01$]。

结论:补肾益智方及各亚组含药血清都能在一定程度上抑制淀粉样 β 蛋白 25-35 对 NG108-15 细胞的损伤作用,但各组情况有所不同。各组含药血清对细胞的保护作用由强至弱依次是去冰片组 > 原方组 > 补肾组 > 益气养血组。故方中冰片的功效有待进一步研究。

主题词: 阿尔茨海默病/中药疗法;淀粉样 β 蛋白;补肾药

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