中文摘要

基于 MAPK 通路探讨人参皂苷 Re、灯盏花素组合物对血管性痴呆大鼠的神经保护作用

目的：

基于丝裂原活化蛋白激酶(mitogen-activated protein kinase,MAPK)信号通路探讨人参皂苷 Re、灯盏花素组合物对血管性痴呆大鼠的神经保护作用，以期为该组合物防治血管性痴呆(vascular dementia, VD)提供实验及理论依据，为中药治疗 VD 提供新的资源与新的研究思路。

方法：

将 64 只大鼠随机分为假手术组，模型组，阳性药组（42mg/kg 血塞通胶囊每日 10ml/kg 体积），组合物低剂量组（人参皂苷 Re 2.5mg/kg+灯盏花素 1mg/kg 每日 10ml/kg 体积），组合物中剂量组（人参皂苷 Re 5mg/kg+灯盏花素 2mg/kg 每日 10ml/kg 体积），组合物高剂量组（人参皂苷 Re 10mg/kg+灯盏花素 4mg/kg 每日 10ml/kg 体积），人参皂苷 Re 组（10mg/kg 每日 10ml/kg 体积），灯盏花素组（4mg/kg 每日 10ml/kg 体积），每组 8 只。除假手术组外，各组大鼠采用双侧颈总动脉永久结扎法制作血管性痴呆大鼠模型。

造模后，假手术组和模型组大鼠给予等体积的纯净水灌胃，其余各组灌胃给药，每日一次，连续 28 天。给予药物后，大鼠的学习以及记忆能力应用 Morris 水迷宫检测，大鼠的海马组织中肿瘤坏死因子 α(TNF-α)、白细胞介素(IL)-6 和白细胞介素(IL)-1β 水平用酶联免疫吸附法(ELISA)法测定，并应用免疫蛋白印迹法(Western blot)法检测细胞外信号调节激酶 1(ERK1)、c-Jun 氨基末端激酶(JNK)、p38 丝裂原活化蛋白激酶(p38MAPK)蛋白在大鼠的海马组织中的表达情况，组织病理 HE 染色，观察海马区组织病理学改变。

结果：

1.Morris 水迷宫结果：

（1）定位航行试验结果：模型组大鼠逃避潜伏期比假手术组显著延长($p < 0.001$)。余下各组均与模型组比较，阳性药组大鼠在定位航行试验第 1 天，逃避

潜伏期差异无显著性。从第 2 天开始，阳性药组大鼠的逃避潜伏期显著缩短(p＜0.01)。组合物低剂量组的大鼠逃避潜伏期仅有一定的降低趋势，但无显著差异。组合物中剂量组以及灯盏花素组大鼠的逃避潜伏期显著缩短(p＜0.05)。人参皂苷Re组大鼠的逃避潜伏期在训练第1天、第2天、第5天明显缩短(p＜0.05)，而第 3 天以及第 4 天两组的逃避潜伏期无明显差异。组合物高剂量组大鼠逃避潜伏期显著缩短（p＜0.001)。

（2）空间探索试验结果：模型组大鼠在训练第 6 天 60s 内穿过平台次数比假手术组明显减少(p＜0.01)。余下各组均与模型组比较，阳性药组和组合物高剂量组大鼠在训练第 6 天 60 秒内穿过平台次数明显增加(p＜0.05)。组合物低剂量组、组合物中剂量组、人参皂苷 Re 组以及灯盏花素组大鼠在训练第 6 天 60 秒内穿过平台次数无显著变化。

2.病理结果：与假手术组比较，模型组大鼠神经元损伤形态变化明显，大脑锥体细胞分布数量减少，细胞胞浆含量下降，核染色质不清；多见锥体细胞发生固缩现象，固缩细胞整体皱缩，胞体染色变深，胞浆和细胞核区分不清。与模型组比较，给药组在不同程度上均可缓解大脑锥体神经元细胞的损伤程度，而其中组合物高剂量组对大脑锥体细胞保护作用效果更为明显，镜下观察可见锥体细胞数量及胞浆清晰丰富，细胞核清晰；部分组织少见细胞发生固缩现象。

3.酶联免疫吸附测定结果：模型组大鼠细胞因子 IL-1β、IL-6、TNF-α 含量均比假手术组显著增加 (p＜0.05)。余下各组均与模型组比较，组合物低剂量组大鼠细胞因子仅 IL-1β 含量显著减少 (p＜0.05)。组合物中剂量组大鼠细胞因子仅 IL-1β、TNF-α 含量减少(p＜0.05)。阳性药组和组合物高剂量组大鼠细胞因子 IL-1β、IL-6、TNF-α 含量均显著减少（p＜0.05)。灯盏花素组与人参皂苷 Re 组各细胞因子含量有一定的减少趋势但无显著差异。

4.免疫蛋白印迹法结果：

（1）ERK1 蛋白含量比较：模型组 ERK1 蛋白表达水平比假手术组显著升高（p＜0.05)。其余各组均与模型组比较，阳性药组、组合物中剂量组、组合物高剂量组 ERK1 蛋白表达水平显著降低（p＜0.001，p＜0.05，p＜0.01)。组合物低剂量组 ERK1 蛋白表达水平无显著差异。

（2）JNK 蛋白含量比较：模型组 JNK 蛋白表达水平比假手术组显著升高

（p＜0.05)。其余各组均与模型组比较，阳性药组、组合物高剂量组 JNK 蛋白表达水平显著降低（p＜0.05)。组合物低剂量组、组合物中剂量组 JNK 蛋白表达水平仅有一定的降低趋势，但差异无显著性。

（3）p38 MAPK 蛋白含量比较：模型组 p38MAPK 蛋白表达水平比假手术组显著升高（p＜0.05)。其余各组均与模型组比较，阳性药组、组合物高剂量组 p38MAPK 蛋白表达水平显著降低（p＜0.05)。组合物低剂量组、组合物中剂量组 p38MAPK 蛋白表达水平仅有一定的降低趋势，但差异无显著性。

结论：

人参皂苷 Re 与灯盏花素组合物可基于 MAPK 信号通路有效改善 VD 大鼠的认知能力，降低 VD 大鼠海马神经元的凋亡，下调 ERK1、JNK、p38MAPK 的表达，抑制炎症因子 IL-1β、IL-6、TNF-α 的生成，对 VD 模型大鼠发挥神经保护作用。

关键词：

人参皂苷 Re 与灯盏花素组合物，血管性痴呆，神经保护，MAPK 信号通路，炎症因子

Abstract

To explore the neuroprotective effect of ginsenoside Re and breviscapine composition on vascular dementia rats based on MAPK signal pathway

Purpose:

To explore the neuroprotective effect of ginsenoside Re and breviscapine composition on vascular dementia rats based on mitogen-activated protein kinase(MAPK) signal pathway, in order to provide experimental and theoretical basis for the prevention and treatment of vascular dementia(VD) by this composition, and provide new resources and new research ideas for the treatment of VD by traditional Chinese medicine.

Methods:

64 stochastic rats were divided into Sham-operated group, model group, Sanqi Panax Notoginseng group (42mg/kg Sanqi Panax Notoginseng capsule 10ml/kg per day), Low-dose combination group (ginsenoside Re 2.5mg/kg breviscapine 1mg/kg per day 10ml/kg per day), Mid-dose combination group (ginsenoside Re 5mg/kg breviscapine 2mg/kg per day 10ml/kg per day), High-dose combination group (ginsenoside Re 10mg/kg breviscapine 4mg/kg per day 10ml/kg per day),Ginsenoside Re group (10mg/kg daily 10ml/kg volume), Breviscapine group (4mg/kg daily 10ml/kg volume),randomly. Each group has 8 rats. Except for the Sham-operated group, the remaining rats were establishing the VD rat model by bilateral permanent ligation of the common carotid artery. After model establishment, rats in Sham-operated group and model group were given equal volume of pure water by gavage, and the other groups were given medicine by gavage once a day for 28 consecutive days.After administration to rats, learning and memory ability of rats was tested by Morris water maze, and tumor necrosis factor-α(TNF-α),interleukin(IL)-6,interleukin(IL)-βwas detected by Elisa.Western

blot was used to determine the expression of Extracellular Regulated Kinase1(Erk1), c-Jun N-terminal kinase(JNK),p38mitogen activate protein kinase(p38MAPK)proteins in the hippocampus of rats from each group. And HE staining was used to monitor the histopathological changes of the hippocampus.

Results:

1. Morris water maze results:

(1)Morris water maze results: Rats in the model group had a longer escape latency compared to the sham-operated group ($p < 0.001$). In the other groups compared to the model group，no significant change in escape latency was observed in the rats of the Sanqi Panax Notoginseng group on day 1 of the localization navigation test. From day 2 onwards, the evasion latency of the rats in the Sanqi Panax Notoginseng group was considerably reduced ($p < 0.01$). There was a tendency to reduce the escape delay in the low-dose group of the combination, but it was not statistically significant. In the Mid-dose combination group and Breviscapine group the escape latency of the rats was shorter ($p < 0.05$). The escape latency of rats in the ginsenoside Re group was considerably reduced on days 1, 2 and 5 of training($p < 0.05$), but on days 3 and 4 there were no significant differences in escape latency between the two groups; The escape latency of rats in the high dose group of the combination was considerably reduced. ($p < 0.001$).

(2) Results of the spatial exploration test: compared with the sham-operated group, the number of times rats crossed the platform within 60 s on the 6th day of training was considerably reduced in the model group ($p < 0.01$). In the other groups, compared to the model group, the number of times the rats crossed the platform within 60 seconds on day 6 of training increased considerably in the Sanqi Panax Notoginseng group and high-dose combination group ($p < 0.05$). No significant changes were seen in the number of times rats crossed the platform within 60 seconds on the 6th day of training in the combination low and medium dose groups, ginsenoside Re group, and lanosterol group.

2. Pathological results: The morphology of hippocampal nerve cells in the model group compared to the Sham group considerably, the number of brain pyramidal cells decreased.The content of cell cytoplasm decreased, and the nuclear chromatin was unclear.Most of the pyramidal cells have pyknosis, the pyknotic cells shrink as a whole, the cell body becomes darker, and the cytoplasm and nucleus are indistinguishable. Compared with the model group, the administration group can alleviate the degree of damage of brain pyramidal neurons in different degrees, and the high-dose group of the composition has more obvious protective effect on brain pyramidal cells. Under the microscope, it can be seen that the number and cytoplasm of pyramidal cells are clear and rich, and the cell nucleus is clear; Pyknosis of cells is rare in some tissues.

3. ELISA results: compared with the sham-operated group, the levels of cytokines IL-1β, IL-6 and TNF-α were considerably higher in the model group ($p < 0.05$). In the remaining groups, IL-1β content was considerably decreased in the low-dose group compared with the model group ($p<0.05$). IL-1β and TNF-α levels were reduced in the mid-dose group of the combination ($p<0.05$). IL-1β, IL-6, and TNF-α contents were considerably decreased in the Sanqi Panax Notoginseng group and the high-dose group of the combination ($p<0.05$). There was a tendency to decrease the content of each cytokine in the lupulin group and the ginsenoside Re group but the difference was not statistically significant.

4. Western blot results：

(1) Comparison of Erk1 protein content: Compared to the sham-operated group, the Erk1 protein expression level was considerably higher and statistically significant in the model group ($p<0.05$). In all remaining groups, Erk1 protein expression was considerably lower in the Sanqi Panax Notoginseng group and Mid-dose combination group and High-dose combination group compared with the model group ($p<0.001$, $p<0.05$, $p<0.01$). The difference in Erk1 protein expression in the low-dose combination group was not statistically meaningful.

(2) Comparison of JNK protein levels: JNK protein expression levels were considerably higher in the model group than in the sham-operated group,which was

statistically significant ($p<0.05$). In the remaining groups, JNK protein expression was considerably lower in the Sanqi Panax Notoginseng group and the high-dose combination group compared with the model group ($p<0.05$). JNK protein expression was reduced in the combination low and medium dose groups, which was not statistically significant.

(3) Comparison of p38MAPK protein levels: p38MAPK protein expression levels were considerably higher in the model group than in the sham-operated group,which was statistically significance ($p<0.05$). Compared with the model group, the expression of p38MAPK protein was considerably lower in the high dose of Sanqi Panax Notoginseng group and combination group ($p<0.05$).Lower expression of the p38MAPK protein was in the Low-dose combination group and Mid-dose combination group, which was not statistically significant.

Conclusions:

The combination of ginsenoside Re and breviscapine can effectively improve the cognitive ability of VD rats based on MAPK signal pathway, reduce the apoptosis of hippocampal neurons in VD rats, down-regulate the expression of ERK1, JNK, p38MAPK, and inhibit the inflammatory factor IL-1 β、 IL-6、TNF- α The production of VD has neuroprotective effect on VD model rats.

Keywords:

Ginsenoside Re and breviscapine composition, vascular dementia, neuroprotection, MAPK signal pathway, inflammatory factors

英文缩写对照表

英文缩写	英文全称	中文名称
VD	vascular dementia	血管性痴呆
MAPK	mitogen-activated protein kinase	丝裂原活化蛋白激酶
ERK	Extracellular Regulated Kinase	细胞外信号调节激酶
JNK	c-Jun N-terminal kinase	c-Jun 氨基末端激酶
p38MAPK	p38 mitogen-activate protein kinase	p38 丝裂原活化蛋白激酶
TNF-α	tumor necrosis factor-α	肿瘤坏死因子 α
IL	interleukin	白细胞介素
NF-κB	nuclear factor kappa-B	核因子 κB
LTP	long-term potentiation	长时程增强
LTD	long-term depression	长时程抑制
NMDA	N-Methyl-D-aspartic acid	N-甲基-D-天冬氨酸
LPS	lipopolysaccharide	脂多糖
Aβ	amyloid-β	淀粉样蛋白-β
caspase-3	caspase-3	半胱氨酸天冬酶-3
Bcl-2	B-cell lymphoma-2	B 淋巴细胞瘤-2
MCP-1	Monocyte Chemoattractant Protein-1	单核细胞趋化蛋白-1

目　录

第 1 章　引言

血管性痴呆(vascular dementia, VD)是一种认知功能障碍综合症，通常由脑血管疾病所引起，它是导致老年痴呆的一个重要原因。血管性痴呆的防治也成为待需解决的公共医疗卫生问题。既往研究表明，VD 的发病机制与氧化应激、炎症反应、细胞凋亡、胆碱能系统缺陷、兴奋性氨基酸毒性等多种病理生理机制相关，但由于 VD 发病机制复杂，现有研究对其具体发病机制尚未完全明确，因此目前的药物治疗效果并不令人满意。目前，中医药研究日益发展，对血管性痴呆的防治具有重要价值。

灯盏花素是一种黄酮化合物，因其可发挥抗血栓形成、抗氧化、抗细胞凋亡[1]、清除氧自由基[2]、抑制血小板聚集等作用，被逐渐应用于抗心脑血管疾病方面的研究[3]。灯盏花素可有效减少炎症细胞因子、降低氧化应激和促凋亡蛋白表达[4]，也已被证实可抑制炎症小体活化触发的细胞焦亡，对脑缺血损伤带来的认知功能障碍具有显著改善作用[5]。

人参在中国被用作传统草药具有悠久的历史，人参皂苷是人参发挥药理作用的主要成分，而人参皂苷 Re 是人参重要的皂苷成分之一。与灯盏花素类似，人参皂苷 Re 可表现出多种作用，其可以发挥抗氧化、抗炎、抗血小板聚集等作用，因此可通过不同的作用机制发挥其药理活性。人参皂苷 Re 的抗炎和抗氧化以及对海马体中脑源性神经营养因子和可塑性相关蛋白的正向调节造成其可发挥神经保护作用对抗认知功能障碍[6]。因此本文考虑人参皂苷 Re 与灯盏花素可能在药效上对防治 VD 具有协同增效作用。

血管性痴呆发病机制尚不完全明确，但炎症相关因子已被证实在其发生发展产生重要作用。炎症相关因子，如 TNF-α、IL-1β、IL-6 等可通过诱导神经元死亡、诱发脑白质病变、破坏血脑屏障等多种途径参与血管性痴呆的发生发展[7]。

在真核细胞中，丝裂原活化蛋白激酶(MAPK)信号转导通路是其主要通路，在细胞的生长、分化、凋亡、炎症过程中起到重大作用[8]。MAPK 信号通路主要分为细胞外信号调节激酶(ERK)，c-Jun 氨基末端激酶(JNK)以及 p38 丝裂原活化蛋白激酶(p38MAPK)等通路，现有研究证明其可通过促进炎症反应、调控海马神经元突触可塑性、调节海马神经元凋亡等方面参与血管性痴呆的发病机制。