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石河子大学

硕士学位论文



枸杞多糖对 ICV-STZ 诱导的痴呆小鼠认知功能 改善作用及机制研究

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**Study on mechanism of LBP to improve cognitive functions of
ICV-STZ-induced Alzheimer's disease mice**

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By

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(Pharmacy)

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摘要

目的:

本实验拟通过侧脑室注射链脲佐菌素 (ICV-STZ) 建立阿尔茨海默病 (AD) 动物模型, 探究枸杞多糖 (LBP) 对痴呆小鼠学习记忆能力的影响及其发挥神经保护作用的相关机制。主要内容有:

(1) 基于 Meta 分析探讨滋补肝肾方药治疗阿尔茨海默病的临床疗效, 并对滋补肝肾方药组分及中药使用频次进行统计。(2) LBP 对 ICV-STZ 诱导的痴呆小鼠学习记忆能力的影响。(3) LBP 对 ICV-STZ 诱导的痴呆小鼠脑组织病理学改变及突触相关蛋白表达水平的影响。(4) LBP 对 ICV-STZ 诱导的痴呆小鼠脑组织 Tau 蛋白磷酸化水平的调节作用及对 IRS1/PI3K/AKT 信号通路相关蛋白表达水平的影响。

方法:

(1) 检索滋补肝肾方药治疗阿尔茨海默病的临床随机对照研究文献, 使用 RevMan 5.1 软件进行 Meta 分析, 并对滋补肝肾方药组分及中药使用频次进行统计。

(2) 15 只 3 月龄雄性 C57BL/6J 小鼠侧脑室注射人工脑脊液 (aCSF) 作为对照组, 即 ICV-aCSF 组; 75 只同月龄小鼠侧脑室注射 STZ 后随机分为 5 组, 每组 15 只, 即模型组 (ICV-STZ), 低剂量枸杞多糖组 (ICV-STZ+L-LBP, 50 mg/kg), 中剂量枸杞多糖组 (ICV-STZ+M-LBP, 100 mg/kg), 高剂量枸杞多糖组 (ICV-STZ+H-LBP, 200 mg/kg), 多奈哌齐组 (ICV-STZ+Donepezil, 0.75 mg/kg)。造模 1 周后, 连续灌胃给药 4 周, 通过 Y 迷宫实验、Morris 水迷宫实验、避暗实验以及跳台实验探究 LBP 对 ICV-STZ 诱导的痴呆小鼠空间认知以及学习能力的影响。

(3) 运用 Nissl 染色观察 LBP 对 ICV-STZ 诱导的痴呆小鼠脑组织形态学的影响。Th-T 染色法评价 LBP 对 ICV-STZ 诱导的痴呆小鼠脑组织 A β 沉积的影响。Western Blot 检测各组小鼠脑组织中突触相关蛋白的表达水平。

(4) Western Blot 检测各组小鼠脑组织中 Ser199、Thr205、Ser396、Ser404 位点的磷酸化 Tau 蛋白水平以及蛋白激酶、蛋白磷酸酶、IRS1/PI3K/AKT 信号通路相关蛋白表达水平。

结果:

(1) Meta 分析结果显示, 滋补肝肾方药治疗 AD 具有明显的临床疗效; 能够明显改善 AD 患者的认知功能; 同时, 滋补肝肾方药可能会改善 AD 患者的生活能力。对 Meta 分析所纳入的文献包含的处方组成及中药频数进行统计, 枸杞的使用频次较高, 枸杞的主要成分枸杞多糖具有良好的降糖、抗氧化、抗衰老、神经保护、提高胰岛素敏感性等作用, 确定 LBP 为动物实验治疗用药。

(2) 在 Y 迷宫实验中, 与 ICV-aCSF 组小鼠相比, ICV-STZ 组小鼠对新异臂的探索次数、探索距离显著减少, 在新异臂中的探索时间也有所降低; 自发交替率显著下降; 与 ICV-STZ 组小鼠相比, LBP 治疗可增加小鼠对新异臂的探索, 自发交替率也显著提高。Morris 水迷宫实验中, ICV-STZ 组小鼠与 ICV-aCSF 组小鼠相比, 第一次穿越平台时间显著延长, 穿越平台次数显著减少, LBP 治疗可显著缩短第一次穿越平台时间并增加穿越平台次数。在避暗实验中, 与 ICV-aCSF 组小鼠相比, ICV-STZ

组小鼠潜伏期缩短、错误次数显著增加；跳台实验中 ICV-STZ 组小鼠潜伏期显著缩短；LBP 治疗后明显延长 ICV-STZ 组小鼠跳台实验潜伏期，并且显著降低了避暗实验和跳台实验错误次数。

(3) Nissl 染色结果显示，ICV-STZ 组小鼠海马各分区及皮层部分神经元缺失严重，神经元胞体缩小，细胞排列稀疏、排布不连续，细胞间隙增大，胞浆尼氏小体减少，染色质分布不均匀。经 LBP 治疗后，神经元缺失有所改善，细胞排列较 ICV-STZ 组小鼠紧密、连续，细胞间隙减小，染色质分布较为均匀。Th-T 染色结果提示，LBP 有减轻 ICV-STZ 组小鼠脑内 A β 的沉积的趋势。Western Blot 结果显示，LBP 可以上调 ICV-STZ 诱导的痴呆小鼠脑组织内 PSD95、Homer-1、SV2A、和 SYP 的蛋白表达水平。

(4) Western Blot 结果表明，LBP 可以降低 ICV-STZ 诱导的痴呆小鼠脑组织 Ser199、Thr205、Ser396、Ser404 位点的磷酸化 Tau 蛋白表达水平，上调 p-GSK3 β (Ser9)蛋白表达水平，下调 p-GSK3 β (Tyr216) 的表达水平，同时，提高了小鼠脑内 IRS1/PI3K/AKT 通路相关蛋白的表达水平。

结论：

(1) 滋补肝肾方药对于 AD 具有良好的临床治疗效果。其中枸杞使用频次较高，LBP 作为枸杞的主要活性成分之一，具有良好的改善胰岛功能、促进胰岛素分泌、神经保护、改善认知等作用，因此确定 LBP 作为动物实验治疗用药。

(2) LBP 对 ICV-STZ 诱导的痴呆小鼠学习记忆功能障碍具有较好的改善作用。

(3) LBP 能够改善 ICV-STZ 诱导的痴呆小鼠脑组织病理损伤，减轻脑内 A β 沉积，提高突触相关蛋白的表达水平。

(4) LBP 可以降低 ICV-STZ 诱导的痴呆小鼠脑组织 Tau 蛋白磷酸化水平，其机制可能与上调 IRS1/PI3K/AKT 通路相关蛋白的表达并调节下游 GSK3 β 蛋白磷酸化相关。

关键词：阿尔茨海默病；胰岛素抵抗；枸杞多糖；链脲佐菌素；Tau 蛋白磷酸化

Abstract

Objective:

In this study, male C57BL/6J mice injected with STZ (3 mg/kg) in the lateral ventricle were used as Alzheimer's disease (AD) animal model. The study aimed to evaluate the effects of Lycium barbarum polysaccharide (LBP) on the learning and memory ability in the model mice and clarify the related mechanisms by which it exerts neuroprotective effects. The main results: (1) Based on Meta-analysis to explore the clinical efficacy of the ZiBuGanShen prescription in the treatment of Alzheimer's Disease (AD), and statistics on the components of the ZiBuGanShen prescription and the frequency of the use of traditional Chinese medicine. (2) Effects of lycium barbarum polysaccharide (LBP) on the learning and memory ability in the ICV-STZ-induced demented mice. (3) The effects of LBP on the brain histopathology and the expression levels of synapse-related protein in ICV-STZ-induced dementia mice. (4) Regulatory effects of LBP on Tau protein phosphorylation in ICV-STZ-induced dementia mice and its effects on the expression levels of proteins related to the IRS1/PI3K/AKT signalling pathway.

Methods:

(1) Randomized controlled trials (RCTS) on treatment of Alzheimer's disease (AD) by ZiBuGanShen prescription were searched, and meta-analysis was performed using RevMan software. After that, statistics on the components of the ZiBuGanShen prescription and the frequency of use of Chinese medicines were also obtained.

(2) Fifteen 3-month-old male C57BL/6J mice were injected with artificial cerebrospinal fluid (aCSF) in the lateral ventricle as a control group (ICV-aCSF group); Seventy-five mice of the same month were injected with STZ in the lateral ventricle and were randomly divided into five groups with 15 mice in each group, the model group (ICV-STZ), the low-dose LBP group (ICV-STZ+L-LBP, 50 mg/kg), the medium-dose LBP group (ICV-STZ+M-LBP, 100 mg/kg), high-dose LBP group (ICV-STZ+H-LBP, 200 mg/kg), and donepezil group (ICV-STZ+Donepezil, 0.75 mg/kg). After 1 week of modelling, the drug was administered by continuous gavage for 4 weeks, and the effects of LBP on the learning and memory ability of ICV-STZ-induced dementia mice were observed by the Y maze test, Morris water maze test, step-through test and step-down test.

(3) The effects of LBP on neurons in the brain tissue of ICV-STZ-induced dementia mice were evaluated by Nissl staining. The effects of LBP on A β desposition in the brain tissue of mice were observed by Th-T staining. The expression level of synapse-associated proteins in the cerebral cortex and hippocampus of mice of each group was determined by Western blot.

(4) Western blot was used to detect the phosphorylation degree of Tau protein at Ser199, Thr205, Ser396 and Ser404 sites as well as the expression levels of protein kinase, protein phosphatase, and protein expression related to IRS1/PI3K/AKT signaling pathway in the brain tissues of mice in each group.

Results:

(1) The results of Meta-analysis show that ZiBuGanShen prescription has significant clinical efficacy in treating AD; it can significantly improve the cognitive function of AD patients; at the same time, it may improve the living ability of AD patients. The composition of ZiBuGanShen prescriptions and the frequency of the traditional Chinese medicines were counted in the literature included in the Meta-analysis. Lycium barbarum polysaccharide was used more frequently, and as one of the main active ingredients of lycium barbarum polysaccharide, LBP has good hypoglycemic, antioxidant, anti-aging, neuroprotective, and insulin sensitivity-enhancing effects. Consequently, LBP was used as a therapeutic agent in animal experiments.

(2) In the Y maze test, compared with the ICV-aCSF group, the exploration times, exploration distance and the time spent in the novel arm were significantly reduced, and the rate of spontaneous alternation was also significantly decreased; After treatment with LBP, the exploration of the novel arm and the rate of spontaneous alternation was significantly increased. In the Morris water maze experiment, mice in the ICV-STZ group had a significantly longer time to the first crossing of the platform and a significantly lower number of crossing times compared with mice in the ICV-aCSF group, and the LBP treatment significantly shortened the time to the first crossing of the platform and increased the number of crossings.

(3) In the step-through test, the latency of ICV-STZ mice was shortened and the number of errors was significantly increased compared with that of mice in the ICV-aCSF group; the latency of mice in the ICV-STZ group was significantly shortened in the step-down test; and LBP treatment significantly prolonged the latency of the step-down test of the mice in the ICV-STZ group, as well as significantly reduced the number of errors in both step-through test and step-down test.

(4) Nissl staining results showed that neuronal deficits were severe in hippocampus and the cortex in ICV-STZ group mice, with neuronal cytosol shrinkage, sparse and discontinuous cell arrangement, increased cell gaps, reduced cytoplasmic Nissl bodies, and non-uniform chromatin distribution. After treatment with LBP, the neuronal deficits were improved, the cell arrangement was tighter and more continuous than that of the ICV-STZ group mice, the cell gap was reduced, and the chromatin distribution was more uniform. Th-T staining results showed that LBP could reduce the deposition of A β in the brain tissue of ICV-STZ-induced dementia mice. The results of Western blot experiment showed that LBP could up-regulate the expression levels of PSD95, SV2A, Homer-1 and SYP in the cortex and hippocampus of ICV-STZ-induced dementia mice.

(5) Western Blot results showed that LBP reduced the phosphorylation of Tau protein at Ser199, Thr205, Ser396, Ser404 sites, up-regulated the protein expression level of p-GSK3 β (Ser9), and down-regulated the expression level of p-GSK3 β (Tyr216), and, at the same time, increased the expression level of the IRS1/PI3K/AKT pathway-related proteins in the brains of ICV-STZ-induced demented mice.

Conclusion:

(1) ZiBuGanShen prescription has a good clinical effect on AD. Lycium barbarum was used more

frequently, and LBP, as one of the main active ingredients of *Lycium barbarum*, has good effects of improving pancreatic islet function, promoting insulin secretion, neuroprotection, and improving cognition, therefore, it was determined that LBP was used as a therapeutic drug in animal experiments.

(2) LBP has a favourable ameliorative effect on learning and memory dysfunction in ICV-STZ-induced dementia mice.

(3) LBP can protect the brain neurons in ICV-STZ-induced dementia mice, reduce the deposition of A β in the brain tissue, and increase the expression level of synapse-associated proteins.

(4) LBP can reduce the Tau protein phosphorylation in the brain of ICV-STZ-induced dementia mice, and the mechanism may be related to the up-regulation of the expression of IRS1/PI3K/AKT pathway-related proteins and the regulation of downstream GSK3 β protein phosphorylation.

Key words: Alzheimer's disease; insulin resistance; *Lycium barbarum* polysaccharide; Streptozotocin; phosphorylated tau protein

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