摘要

研究背景：阿尔茨海默症（Alzheimer's disease，AD）是一种起病隐匿且进行性发展的慢性神经系统退行性疾病，以记忆障碍、语言功能和其他认知能力衰退为主要症状，可导致患者日常生活能力下降，出现精神行为异常，给家庭和社会带来极大的负担。全球 AD 患者人数已高达 5000 万，预计 2050 年将增加至 15200 万。AD 已成为全球第五大死因，因此，寻找改善 AD 的药物，是目前科研人员亟待解决的主要问题。

FOXO 家族与 AD 的发生有着密切的关系。β淀粉样蛋白（Aβ）斑块刺激可以导致 FOXO3a 的一系列翻译后修饰和核移位，并通过提高 BIM 表达来诱导神经元的氧化应激和细胞凋亡，最终导致神经元的丢失并引起一系列的神经退行性疾病。因此，FOXO3a/BIM 可能是治疗阿尔茨海默症的一个潜在的位点。

小檗碱（分子式为 $C_{20}H_{19}NO_5$，分子量为 353.36 g/mol）是各种小檗属植物茎和根的主要异喹啉生物碱成分。小檗碱在传统的阿育吠陀和中药系统中有广泛的应用。它具有良好的药理活性，包括镇痛、抗炎、抗癌、降糖、抗高脂血症、心脏保护、增强记忆、抗抑郁、抗氧化、抗伤害、抗菌、抗 HIV 和降胆固醇作用。在课题组前期研究中，我们发现小檗碱不仅可以增强海马神经元的抗凋亡能力，同时也对糖尿病脑病的认知功能障碍具有改善作用。近年来研究也证明了小檗碱作为神经保护剂的显著效果。但小檗碱对阿尔茨海默症的治疗作用及其作用尚需要进一步明确。

研究目的：通过体内外实验研究小檗碱是否能通过 FOXO3a/BIM 通路改善阿尔兹海默症中的认知功能障碍和神经元损伤，以及探讨其相关机制。

方法与结果：

1. 体内外水平明确小檗碱对 AD 认知功能障碍和神经元损伤的治疗作用

利用 D-gal 与 $AlCl_3$ 构建的 AD 小鼠模型，通过莫里斯水迷宫检测小鼠认知功能改善情况。处死并提取小鼠脑组织，进行 HE、尼氏及 TUNEL 染色观察小鼠脑内神经元变性及凋亡情况。结果表明，小檗碱可以明显降低小鼠的逃逸潜伏期，改善小鼠的认知功能障碍，并减轻 AD 小鼠脑内的神经元凋亡和变性。随后，

又利用 HT22 神经元细胞与 Aβ25-35 构建体外 AD 细胞模型，进行 MTT 细胞活力检测筛选 Aβ25-35 造模浓度和小檗碱给药的安全剂量及有效剂量。结果发现，40μM 浓度的 Aβ25-35 可以使 HT22 细胞活力下降至 70%左右，符合造模要求。在 1μM 浓度以下的小檗碱对 HT22 细胞没有明显杀伤效果。小檗碱在安全剂量范围内可以以剂量依赖性的方式提高 HT22 细胞的细胞活力，且 0.2μM 浓度的小檗碱改善作用最好，因此在后续实验中选择了 40μM 浓度的 Aβ25-35 进行造模和 0.2μM 浓度的小檗碱给药。

2.小檗碱通过 FOXO3a/BIM 信号通路治疗 AD

通过网络药理学筛选获得小檗碱与 AD 具有潜在的结合位点共 92 个，随后通过蛋白-蛋白互作分析，KEGG 通路富集与 GO 功能富集筛选与 AD 最相关的前 20 个靶点，其中，我们发现 FOXO3a 与 PI3K/AKT 通路在小檗碱和 AD 交联时具有较高的相关性。而在进一步的文献阅读中，FOXO3a 与神经元细胞的凋亡有着密不可分的关系。

为了验证 FOXO3a/BIM 通路是否参与了小檗碱治疗 AD 的过程，首先，我们提取了小鼠动物模型的脑组织蛋白并利用 WB 技术检测了 FOXO3a/BIM 蛋白表达情况的改变。结果发现在模型组中 FOXO3a 磷酸化水平降低，BIM 表达增高；小檗碱组中 FOXO3a 磷酸化水平升高，BIM 表达降低。证明小檗碱可以在体内水平增加 AD 动物模型中 FOXO3a 的磷酸化水平并降低 BIM 蛋白表达。

随后，我们又提取 HT22 细胞 AD 模型的细胞蛋白进行了 WB 实验。与体内实验结果类似，在细胞蛋白 WB 实验中，Aβ25-35 刺激后，细胞模型中的 FOXO3a 的磷酸化水平被显著抑制，而 BIM 的表达显著提高。小檗碱给药则增加了 FOXO3a 的磷酸化水平并降低了 BIM 的表达。FOXO3a 在磷酸化水平下降后会导致其核易位程度增加。随后，为了观察小檗碱对 FOXO3a 核易位程度的影响，我们继续使用 AD 细胞模型进行免疫荧光实验，结果表明在 Aβ25-35 刺激下，FOXO3a 的核定位水平增加，小檗碱则降低了核易位程度。通过体内外实验我们发现，FOXO3a/BIM 通路可能是 AD 治疗的一个有效靶点。

3.小檗碱可以通过影响 PI3K/AKT 通路来调控 FOXO3a/BIM 信号通路

PI3K/AKT 通路是 FOXO3a/BIM 通路的直接上游，PI3K 的去磷酸化会导致 AKT 和 FOXO3a 的去磷酸化。因此，我们在体外水平使用 PI3K 抑制剂 LY294002

和或小檗碱给药并提取蛋白进行 WB 实验。随后检测了 p-AKT/AKT、p-FOXO3a/FOXO3a、BIM 的表达水平改变。结果表明，在模型组中，与对照组相比， p-AKT/AKT 和 p-FOXO3a/FOXO3a 水平显著降低，BIM 蛋白的表达显著升高。在小檗碱给药组中，p-AKT/AKT 和 p-FOXO3a/FOXO3a 水平的下降被显著逆转，BIM 表达水平明显降低。但在抑制剂组中， p-AKT/AKT 和 p-FOXO3a/FOXO3a 水平被再次下调，BIM 蛋白的表达也明显升高，小檗碱的治疗效果被 LY294002 消除，证明小檗碱可能通过影响 PI3K/AKT 通路来调控 FOXO3a/BIM 信号通路。

结论：小檗碱可以通过 FOXO3a/BIM 信号通路治疗阿尔兹海默症中的认知功能障碍和神经元损伤。

关键词：阿尔茨海默症；小檗碱；神经元；凋亡；FOXO3a；BIM

Abstract

Backgrounds: Alzheimer's disease (AD) is a chronic neurodegenerative disease with insidious onset and progressive development, with memory impairment, language function and other cognitive decline as the main symptoms, which can lead to a decline in the patient's daily life abilities, mental and behavioral abnormalities, and bring great burden to the family and society. The number of AD patients worldwide has reached 50 million and is expected to increase to 152 million by 2050. AD has become the fifth leading cause of death in the world, so finding drugs to improve AD is a major problem that researchers need to solve urgently.

The FOXO protein family is closely related to the occurrence of AD. After stimulation of β amyloid (Aβ), it causes apoptosis of neuronal cells. When the FOXO3a protein is overexpressed, it leads to oxidative stress and apoptosis of neurons, eventually leading to the loss of neurons and causing a series of neurodegenerative diseases. Aβ can lead to a series of post-translational modifications and nuclear localization of FOXO3a and induce cell death through promote BIM expression. Therefore, FOXO3a may be a potential target for the treatment of Alzheimer's disease.

Berberine (molecular formula $C_{20}H_{19}NO_5$, molecular weight 353.36 g/mol) is the main isoquinoline alkaloid component of the stems and roots of various berberine plants. Berberine has a wide range of applications in traditional Ayurvedic and Chinese medicine systems. It has good pharmacological effect, including analgesic, anti-inflammatory, anticancer, hypoglycemic, antihyperlipidemia, cardioprotective, memory-enhancing and antidepressant, antioxidant, anti-harm, antibacterial, anti-HIV and cholesterol-lowering effects. In the previous research of our lab, we found that berberine can not only enhance the anti-apoptotic ability of hippocampal neurons, but also have an improving effect on cognitive dysfunction in diabetic encephalopathy. Therefore, the effect of berberine in improving cognitive dysfunction can be confirmed. At the same time, in bioinformatics analysis, after intersecting berberine and AD targets, a total of 92 common targets were found. Among them, the most relative proteins is the FOXO3a. Therefore, we can hypothesize that berberine may improve cognitive dysfunction and neuronal damage in AD through the FOXO3a

pathway.

Objective：This paper will use the constructed in vivo and in vitro AD models to investigate whether berberine can improve cognitive dysfunction and neuronal damage in Alzheimer's disease through the FOXO3a pathway, and explore its related mechanisms.

Methods and results:

1.The therapeutic effect of berberine on AD cognitive dysfunction and neuronal damage on vivo and vitro.

The AD mice model constructed by D-gal and $AlCl_3$ was used to detect the improvement of cognitive function through the Morris water maze. Mice were sacrificed and brain tissues were extracted. The results showed that berberine could significantly reduce the escape latency of mice and improve cognitive dysfunction in mice. Then, HE, Nissel and TUNEL staining, were performed to detect apoptosis and FOXO3a/BIM pathway protein expression in mice, and we found the apoptosis level is decreased by BBR. Later, we used HT22 cells and Aβ25-35 to construct vitro cell model, and used MTT assay to detect safe and effective concentration of berberine. The results showed that 40μM concentration of Aβ25-35 could reduce the viability of HT22 cells to about 70%, which met the requirements of modeling. Berberine below 1 μM concentration has no obvious killing effect on HT22 cells, berberine can improve the cell viability of HT22 cells in a dose-dependent manner within 1 μM, and berberine has a most effective therapeutic effect at the concentration of 0.2 μM, so 40 μM concentration of Aβ25-35 was selected for modeling and 0.2 μM concentration of berberine administration was selected in subsequent experiments.

2. Berberine improves AD through the FOXO3a/BIM signaling pathway.

A total of 92 potential binding sites between berberine and AD were obtained through network pharmacological screening, and then through protein-protein interaction analysis, KEGG pathway enrichment and GO function enrichment screened the top 20 targets most relevant to AD, among which we found that PI3K/AKT pathway and FOXO3a had a high correlation when berberine and AD were crosslinked. In further literature reading, FOXO3a is inextricably linked to apoptosis of neuronal cells. Immunofluorescence results showed that the level of nuclear localization of FOXO3a was increased upon Aβ25-35 stimulation, while berberine improved the level of nuclear translocation.

To verify whether the FOXO3a/BIM pathway is involved in the process of berberine treatment in AD, Firstly, we extracted brain tissue proteins from mouse animal models and examined the alteration of FOXO3a/BIM protein expression using WB method. The results found that the phosphorylation of FOXO3a decreased and BIM expression increased in the model group; the phosphorylation of FOXO3a increased and BIM expression decreased in berberine group, demonstrated that berberine can increase phosphorylation of FOXO3a and decrease BIM protein expression in AD animal models.

Subsequently, we extracted cellular proteins from HT22 cell AD model. Similar to the in vivo results, the phosphorylation of FOXO3a was significantly suppressed in Aβ25-35 after stimulation. Berberine administration increased the phosphorylation level of FOXO3a and decreased the expression of BIM. FOXO3a After decreased phosphorylation leads to an increased degree of its nuclear translocation. After FOXO3a decreased phosphorylation leads to an increased level of its nuclear translocation. Subsequently, to observe the effect of the level of nuclear translocation of berberine FOXO3a, we used immunofluorescence experiments in the AD cell model and showed that the level of nuclear localization of FOXO3a increased upon Aβ25-35 stimulation, while berberine decreased the level of nuclear translocation. Through in vitro and in vivo experiments, we found that the FOXO3a/BIM pathway might be an effective pathway for AD treatment.

3. Berberine can regulate FOXO3a/BIM signaling pathway by affecting the PI3K/AKT pathway.

The PI3K/AKT pathway is directly upstream of the FOXO3a/BIM pathway, and dephosphorylation of PI3K causes dephosphorylation of both AKT and FOXO3a. Therefore, we used the PI3K inhibitor LY294002 and/or BBR and then extracted protein for WB assay. Subsequently, the expression levels of p-AKT/AKT, p-FOXO3a/FOXO3a, and BIM were detected. The results showed that in the model group, the levels of p-FOXO3a/FOXO3a and p-AKT/AKT were significantly reduced and the expression of BIM protein was significantly increased compared with the control group. In the berberine administration group, the decreases of p-AKT/AKT and p-FOXO3a/FOXO3a levels were significantly reversed, and BIM expression levels were significantly reduced. In the inhibitor group, the levels of p-FOXO3a/FOXO3a and p-AKT/AKT were downregulated again, the expression of

BIM protein was also significantly increased, and the therapeutic effect of berberine was eliminated by LY294002, demonstrated that berberine regulates the FOXO3a/BIM signaling pathway by affecting the PI3K/AKT pathway.

Conclusion: Berberine can improve cognitive dysfunction and neuronal damage in Alzheimer's disease through the FOXO3a/BIM signaling pathway.

Key words: Alzheimer's disease; Berberine; Neuronal cell; Apoptosis;FOXO3a; BIM

目 录

英文缩写表

缩略词	英文全称	中文全称
NDD	Neurodegeneration diseases	神经退行性疾病
AD	Alzheimer's disease	阿尔茨海默症
fAD	familial Alzheimer's disease	家族性阿尔茨海默症
sAD	Sporadic Alzheimer's disease	散发性阿尔茨海默症
ALS	Amyotrophiclateralsclerosis	肌萎缩侧索硬化
HDD	Huntington'sdisease	亨廷顿病
PD	Parkinson's disease	帕金森病
BBR	Berberine	小檗碱
BBB	Blood-Brain Barrier	血脑屏障
Aβ	β-Amyloid	β淀粉样蛋白
APOE	Apolipoprotein E	载脂蛋白 E
PSEN	Presenilin	早老素/早老蛋白
NFT	Neurofibrillary Tangle	神经纤维缠结
BACE1	Beta-Secretase 1	β-分泌酶 1
sAPPα	Soluble peptide APPα	分泌型 APPα
sAPPβ	soluble peptide APPβ	分泌型 APPβ
APP	Amyloid precursor protein	淀粉样前体蛋白
IL-6	interleukin-6	白介素-6
IL-1 β	interleukin-1β	白介素-1β
TNF-α	tumor necrosis factor-α	肿瘤坏死因子-α
iNOS	Inducible Nitric oxide	诱导型一氧化氮合酶
ROS	Reactive Oxygen Species	活性氧
FOXO	Forkhead Box O	叉头盒蛋白 O
NGF	Nerve growth factor	神经生长因子