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Original Article

STUDY ON THE SYNTHESIS OF PUERARIN DERIVATIVES AND THEIR ANTI-VASCULAR DEMENTIA ACTIVITY

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ABSTRACT

In this study, puerarin derivatives were designed by adding an active acetonitrile group that inhibits cyclooxygenase-2 (COX-2) in order to enhance the anti-vascular dementia drug activity. The acetonitrile group was linked to puerarin at the 7/4 'positions by a phenolic hydroxyl to give 7-mono-and 7, 4' di-substituted derivatives of puerarin. These structures were confirmed by ^{1}H NMR spectroscopy and MS spectroscopy. We compared the affinity of puerarin derivatives and puerarin for cyclooxygenase-2 (COX-2) using molecular docking. In addition, the anti-vascular dementia activity of the developed puerarin derivatives was studied by water maze, novel object recognition, and the determination of inducible nitric oxide synthase (iNOS) enzyme activity at the cerebral cortex of mice. Experimental results showed that the puerarin derivatives have a good affinity for COX-2 with therapeutic effects against vascular dementia. The results of this study suggest that the protective effects of the puerarin derivatives against vascular dementia may be related to suppression of inflammation associated with ischemia-reperfusion injury through inhibition of COX-2.

Keywords: Puerarin, COX-2, Vascular dementia

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INTRODUCTION

Vascular dementia (VD) is an acquired, persistent mental retardation syndrome caused by the damage of cerebral parenchyma. VD includes ischemic, hemorrhagic, acute and chronic hypoxic cerebrovascular diseases. Among them, the ischemic cerebrovascular disease is thought to play a particularly important role in the pathogenesis of VD. In recent years, a large number of reports confirmed that diffuse inflammatory mediators during ischemia-reperfusion injury contribute to the pathogenesis of VD. In addition, cyclooxygenase-2(COX-2) and its metabolites participate in the process of cerebral ischemia-reperfusion injury by causing damage to the vascular system, blood-brain barrier and neurons that play an important role in inflammation of the nervous system [1].

Puerarin is an isoflavone extracted from dried roots of Pueraria thomsonii Benth. Its chemical name is 8-β-D-glucopyranoside-4'-7- dihydroxyflavone (molecular formula C21H20O9, the relative molecular mass of 416) and it is one of the active ingredients in the traditional Chinese medicine, Pueraria lobata. Studies have shown that puerarin can inhibit neutrophil infiltration in the brain tissue of rats with cerebral ischemia-reperfusion [2], inhibit the expression of COX-2 in the brain tissue of mice with VD, decrease the concentration of cytokines such as TNF-α, IL-1β, and IL-6 in brain tissue following ischemia-reperfusion, reduce the aggregation of leukocytes at the lesions, and organize localized inflammatory cascade reactions [3, 4].

MATERIALS AND METHODS

Reagents and instruments

MS spectra were obtained with an Agilent G6300 Series (HPLC-MS) LC-MS analyzer. ^{1}H NMR spectra were obtained with a Bruker DMX600MHz/300MHz NMR spectrometer (DMSO-d6 as solvents, tetramethylsilane as internal standard-). Auto Dock Vina molecular docking software was used. Puerarin pharmaceutical raw materials (≥98%) were purchased from Xi'an Frierson Biotechnology Co., Ltd. All reagents and solvents were of analytical grade. The silica gel used for column chromatography (reagent grade, 200 to 300 mesh) was purchased from Qingdao Haiyang Chemical Co., Ltd.

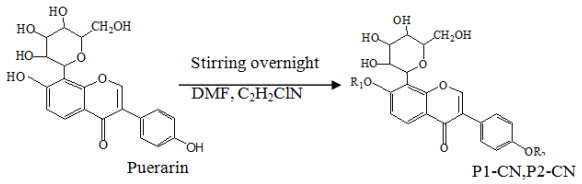
Experimental animals

Male Kunming mice (SCXK (Army) 2014-012, weighing 22 ~ 28g) were used in this study.

RESULTS AND DISCUSSION

Synthesis of puerarin derivatives (scheme)

In this study, puerarin derivatives were designed by adding an active acetonitrile group that inhibits COX-2 in order to enhance its anti-vascular dementia drug activity. The acetonitrile group was linked to puerarin at the 7/4 'positions by a phenolic hydroxyl to give 7-mono-and 7, 4' di-substituted derivatives of puerarin. This process resulted in the synthesis of two compounds that were previously unreported. The route of synthesis is shown in Scheme 1. We evaluated the affinity of these two derivatives towards COX-2 as well as their anti-vascular dementia activities.



Scheme 1: Synthesis route of puerarin derivatives, R_1,R_2:C_2H_2N,C_2H_2N (P_1-CN);C_2H_2N,H (P_2-CN)

The puerarin (15g, 36 mmol) was dissolved in anhydrous dimethylformamide (DMF; 200 ml) followed by the addition of K_2CO_3 (29.8g, 216.3 mmol) at room temperature. The mixture was stirred for 90 min followed by the addition of chloroacet onitrile (16.22g, 216 mmol), and then stirred again at room temperature overnight. The mixture was filtered to remove insoluble solid matter, and spun to oil state, and then ground with dry ether. The ether solution was poured off and the remaining precipitate spun dried, then dissolved in anhydrous methanol. Silica gel column chromatography (DCM: MeOH = 40: 1 ~ 15: 1 ~ 10: 1) was then used to isolate the two products.

P_1-CN: M+H495.1H-NMR(300MHz,DMSO)δ: 8.56(1H,d,J=5.4Hz), 8.18 (1H,dd,J=9.0Hz,J=2.7Hz),7.62(1H,m),7.37(1H,m),7.15(2H,dd,J=9.0Hz, J=2.1Hz), 5.38 (1H,s), 5.28 (1H,s), 5.23(2H,s), 5.06 (1H,d,J=39Hz), 4.76-5.02(3H,m), 4.45(1H,m), 4.08(1H, m), 3.74(1H,m), 3.43(1H,m), 3.24-3.28(3H,m).

P2-CN: M+H457.1H-NMR(300MHz,DMSO)δ: 9.56(1H,d,J=4.2Hz),8.46 (1H,d,J=7.5Hz),8.16(1H,dd,J=9.0Hz,J=2.4Hz),7.41(2H,dd,J=8.7Hz,J=3. 3Hz),7.32-7.37(1H,m),6.81(2H,dd,J=8.4Hz,J=1.8Hz),5.37(1H,s),5.27(1H,s),4.75-5.06(4H,m),4.04(1H,m),3.74(1H,m),3.42(1H,m),3.14-3.24 (3H,m).

Molecular docking of puerarin derivatives and COX-2

Molecular docking is a mutual recognition process by geometric and energy matching between two or more molecules. Molecular docking relies on the interactions that occur between enzymes, enzyme activators, enzyme inhibitors, drug molecules, small molecules (ligands) and target enzymes (receptors)that are mutually bound to each other [5, 6]. Molecular docking calculations are used to evaluate the quality of ligand-receptor interactions and to find the best binding mode of two molecules according to the principles of complementary geometry, energy, and the chemical environment when the ligand molecules are at the active site of the receptors.

In this study, the structure of the target protein, COX-2, was taken from the Protein Data Bank (PDB). The ligand structures used were of puerarin and the puerarin derivatives P_1-CN and P_2-CN. Using the Auto Dock Vina software, the COX-2 protein structure was first dehydrated then hydrogenated to dock with the target ligand and the binding free energy was calculated. The results are shown in table 1. Using this information, we determined that the affinity of the puerarin derivatives towards COX-2 was higher than or similar to that of puerarin itself, indicating that the derivatives possess good COX-2 inhibitory properties. This, in turn, suggested that the puerarin derivatives have anti-vascular dementia activity.

Table 1: Docking of COX-2 and puerarin derivatives

Ligand	Affinity(kcal/mol)
Puerarin	-9.7
p1-CN	-10.1
P2-CN	-9.6

Anti-vascular dementia activity of puerar in derivatives

The vascular dementia mouse model was established by bilateral carotid artery ligation and reperfusion. Male Kunming mice were used in this study. The animals were randomly divided into seven groups (10 mice in each group): sham group, model group, positive puerarin at 100 mg/kg, puerarin derivative P_1-CN at 25 mg/kg or 100 mg/kg, puerarin derivative P_2-CNat 25 mg/kg or 100 mg/kg. Biological anti-vascular dementia activity tests were conducted in the dementia mouse model, including the water maze [8], novel object recognition, and determination of iNOS enzyme activity at the cerebral cortex [9] following treatment with puerarin or the puerarin derivatives P_1-CN and P-CN. All data are represented as x±SD and were analyzed by ANOVA using the statistical software SPSS15.0 [10]. $p<0.05$ was considered to be statistically significant.

The model group required longer periods of time to swim to the platform in the water maze tests compared to the sham group (fig. 1), indicating that the VD model was properly established. Compared with the model group, the swimming time of mice in the puerarin derivative P_1-CN at 100 mg/kg and P_2-CN at 100 mg/kg groups was shorter. In addition, the swimming time of mice in the puerarin derivative P_1-CN at 100 mg/kg group was significantly reduced ($P<0.05$), demonstrating therapeutic efficacy against cerebral ischemia-mediated vascular dementia.

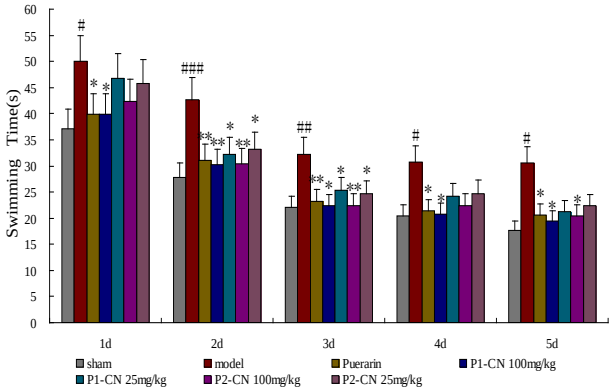


Fig. 1: Effect of P_1-CN and P_2-CN on swimming time in water maze test in mice with cerebral ischemia（*n*=10, $\bar{X}$±*SD*）

#P<0.05,##P<0.01,###P<0.001vs. Sham group; *P<0.05, **P<0.01vs. model group

Table 2: Effect of P_1-CN and P_2-CN on discrimination index for the new object of 1h and 24h in the novel object recognition in mice with cerebral ischemia（*n*=10, $\bar{X}$±*SD*）

Group	Dose (mg/kg)	Discrimination index (1h)	Discrimination index (24h)
Sham	—	0.27±0.28	0.45±0.51
Model	—	-0.29±0.22 #	-0.31±0.43 ##
Puerarin	100	0.21±0.64*	0.04±0.20
P1-CN	100	0.19±0.17*	0.24±0.44*
	25	-0.13±0.40	-0.01±0.49
P2-CN	100	0.14±0.42*	0.08±0.45*
	25	-0.11±0.51	-0.29±0.68

#P<0.05,##P<0.01 vs. sham group; *P<0.05 vs. model group.

The novel object recognition experiment is a learning and memory test based on the innate explorative tendency of animals [11, 12]. Since this method allows mice to learn and acquire memory under a free active state, its simulates human learning and memory. Additionally, not only do the flexible changes of new objects (shape, size) allow us to study the mechanisms of long-term or short-term memory formation in animals, it also facilitates the evaluation of memory formation at a particular stage. Hence, the novel object recognition method is widely used in areas such as learning, memory, and evaluation of new drugs. The results in table 2 show that the discrimination index of the model group at 1h and 24h was significantly lower than that of the sham group, indicating that this model was properly established. Compared with the model group, the discrimination index of both the puerarin derivative P_1-CN at 100 mg/kg and P_2-CN at 100 mg/kg groups at 1h and 24h increased significantly, suggesting that they have therapeutic effects on cerebral ischemia-induced vascular dementia.

Inducible nitric oxide synthase (iNOS) and COX-2 are two important proteins in the NF-κB signaling pathway that work in coordination and are key proteins in the process of inflammation [13]. The high expression of iNOS in rat brain after ischemia and reperfusion may be related to inflammation and oxidative stress [14, 15]. The results in fig. 2 show that the activity of iNOS in the model group was higher than that of the sham group. Compared with the model group, the activity of iNOS in the puerarin derivative P_1-CN at 100 mg/kg group and puerarin group decreased significantly. These results indicate that the puerarin derivative P_1-CN at 100 mg/kg had a therapeutic effect on cerebral ischemia-induced vascular dementia that may be associated

with inhibition of a series of inflammatory reactions induced by ischemia-reperfusion.

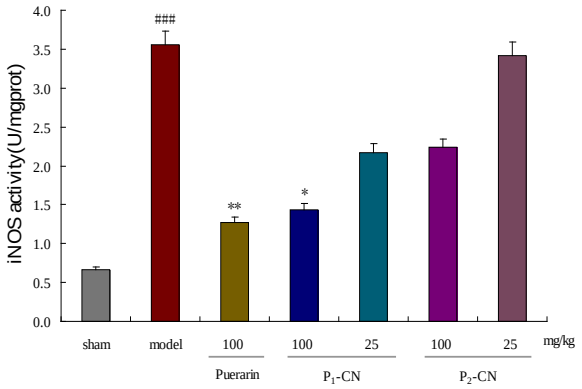


Fig. 2: Effect of P_1-CN and P_2-CN on the activity of iNOS in hippocampus of mice with cerebral ischemia（*n*=10, $\bar{X}\pm SD$

###P<0.001vs. sham group; *P<0.05, **P<0.01vs. model group

CONCLUSION

In this study, an acetonitrile group was linked to puerarin at the 7/4 'position by a phenolic hydroxyl to give 7-mono-and 7, 4' di-substituted derivatives of puerarin two previously unreported compounds. The results from the ^{1}H NMR spectra, water maze test, novel object recognition, and determination of iNOS enzyme activity indicate that these two derivatives had a greater affinity towards COX-2 and possessed anti-vascular dementia activity compared to the parent compound (puerarin). The mechanism may be related to inhibition of inflammation associated with ischemia-reperfusion.

CONFLICT OF INTERESTS

Declared none

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