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RESEARCH ARTICLE

Flavone from Zhongjiefeng (*Herba Sarcandrae Glabrae*) inhibits platelet apoptosis in immune-induced bone marrow failure through mitochondrial pathway

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Abstract

OBjECTIVE: To investigate the effect of Flavone from Zhongjiefeng (*Herba Sarcandrae Glabrae*) on the platelet number in immune-induced bone marrow failure (BMF) and its mechanism of mitochondrial apoptotic pathway.

METHODS: Immune-induced BMF model, established in mice, was randomly divided into four groups: normal control group without BMF, BMF control group, cyclosporine (CSA) group and flavone group (n = 10 in each group). Mice were given 0.027 g/kg cyclosporine or 0.2 g/kg flavone lavage daily in either the cyclosporine or flavone group respectively. Platelet count, mitochondrial transmembrane potential (ΔΨm), cytochrome C (Cyt C), phosphatidylserine (PS), changes of calcium ion (Ca^{2+}), and protein expression of mitochondrial apoptotic pathway including B-cell lymphoma-2 (bcl-2) Homologous Antagonist-Killer Protein (Bak), bcl-2-associated X protein (Bax), caspase-3, caspase-8, and caspase-9 were examined and compared.

RESULTS: Compared with the normal control group, the BMF group had significantly lower levels of platelet count, ΔΨm, and expressions of caspase family proteins as well as higher levels of Cyt C, PS, Ca^{2+}, and expressions of Bak and Bax (all $P < 0.05$). Compared with the BMF group, the CSA and flavone groups had significantly higher ΔΨm and expressions of caspase family proteins (all $P < 0.05$) whereas the levels of Cyt C, PS, Ca^{2+}, and expressions of Bak and Bax were reduced (all $P < 0.05$). More importantly, the flavone group had higher levels of Cyt C, Ca^{2+} and expressions of Bak and Bax compared with the CSA group (all $P < 0.05$), while the levels of PS and caspase family proteins were reduced (all $P < 0.05$).

CONCLUSION: Flavone from Zhongjiefeng (*Herba Sarcandrae Glabrae*) significantly increases the platelet number and prevents its apoptosis through mitochondrial pathway.

Keywords: Flavone; Herba Sarcandrae; Mitochondria; Apoptosis regulatory proteins

INTRODUCTION

Bone marrow failure (BMF) is a type of diseases characterized by reduced blood cell regeneration capability,[1] especially the platelet regeneration, which threatens life. Excessive platelet apoptosis is one of the pathogenic causes for immune-induced BMF. Mitochondrial apoptotic pathway is one of the important pathways modulating platelet apoptosis. The mechanisms include early manifestation of reduction in mitochondrial transmembrane potential ($\Delta\Psi m$) and formation of mitochondrial permeability transition pore (MPTP) followed by multi-transportation of mitochondrial proteins to cytoplasm to exert a pro-apoptotic effect. Pro-apoptotic proteins translocate to mitochondria and cytochrome C releases from mitochondrial intermembrane space to cytoplasm. In platelet, cysteinyl aspartate specific proteinase (caspase)-8, caspase-9 and caspase-3 are activated subsequently, hence splitting cytoskeleton. The membrane phosphatidylserine (PS) is then exposed extracellularly and the platelet then undergoes shrinking, dropping of microparticles and apoptosis.[2] The commonly-used medication for thrombocytopenia is cyclosporine (CSA), which exerts its effect through inhibiting apoptosis by mitochondrial pathway.[3] As found out by our previous pilot study, mitochondrial pathway inducing platelet apoptosis could lead to abnormal blood coagulation and hemorrhage.[4]

Zhongjiefeng (*Herba Sarcandrae Glabrae*) is the dried whole-plant of Chloranthaceae Caoshanhu and a type of commonly-used Traditional Chinese Medicine (TCM) with its effect on "promoting blood circulation, eliminating mass and relieving swelling, and cooling blood and hemostasis". Clinically, it has been prescribed for hemorrhagic disease caused by thrombocytopenia. Current study has found out the major chemical composition of Zhongjiefeng (*Herba Sarcandrae Glabrae*) includes sesquiterpenes (atractylenolide-II, III, IV, and chloranthalactone A, B, E, F and G), flavone (isoliquiritigenin, isoliquiritin and 7-O-methyl naringenin), coumarins (isofraxidin, fraxidin and fraxin), organic acids (fumarate, succinic acid and chlorogenic acid) and volatile oils.[5] Total flavonoids from Zhongjiefeng (*Herba Sarcandrae Glabrae*) is the effective component. Previous mouse research has demonstrated its effect on promoting proliferation of megakaryocytic series,[6] improving the post-chemo therapy thrombocytopenia,[7] increasing white blood cells and platelet count.[8] However, its mechanism has not been elucidated. It still remains unknown whether its effect on increasing platelet count is through inhibiting mitochondrial apoptotic pathway. Therefore, we aim to investigate the effect of flavone from Zhongjiefeng (*Herba Sarcandrae Glabrae*) on the platelet number in mice with immune-induced bone marrow failure and elucidate whether its effect is through mitochondrial apoptotic pathway.

MATERIALS AND METHODS

Animal

Forty C57BL/6 mice (20 male and 20 female mice) aged from 8 to 12 weeks, weighing (20 ± 2) g were purchased from Shanghai Slac Laboratory Animal Corporation (Shanghai, China). Mice were housed in the animal center of the company with free access to water and food. The animal approval number was SCXK 2012-0002. The study was approved by the experimental animal ethics commitee of Shanghai Baoshan hospital of Integrated Traditional Chinese and Western Medicine.

Flavone and cyclosporine preparation

Flavone from Zhongjiefeng (*Herba Sarcandrae Glabrae*) is isolated and purified by using HPD400 macroporous adsorptive resin.[9] Its characteristic figure by high performance liquid chromatography (HPLC) is presented in Figure 1. Cyclosporine (S0408, 25 mg × 50 tablets, Novartis Pharma, Freiburg Area, Germany) is made into 4 mg/mL solution with sterile saline. Lavage solution is then diluted to 0.1 mL per 10 grams of mouse weight.

Bone marrow failure animal model

The animal model of bone marrow failure is established by following method from Liu *et al.*[10] Briefly, thymus glands were dissected from DBA/2 mice sacrificed by cervical dislocation, filtered through Nylon filters and passed through the size-4 syringe needle to form single-cell suspension. Trypan Blue staining was used to assess the cell viability, hence determining the cell number. A total of 1×10^6 cells were then administered through the tail vein of a C57BL/6 mouse for 4 h after exposure to ^{60}Co-γ radiation [5.5Gy (1.1 Gy/min × 5 min)]. Three days after modeling, peripheral blood was drawn from the mouse-tail vein and detected by the automatic blood cell analyzer. When pancytopenia was shown, it suggested that the model was successful.

Study design

Forty C57BL/6 mice were randomly assigned to four groups with 10 mice in each group: normal control group, BMF control group, CSA group and flavone group. Mice of the normal control group were healthy C57BL/6 mice without BMF modeling. Mice of the BMF control group were exposed to radiation and cell transfusion and had no treatment with either CSA or flavone. Mice of the CSA group received daily lavage with 0.027 g/kg (0.1 mL/10 g) of CSA whereas the