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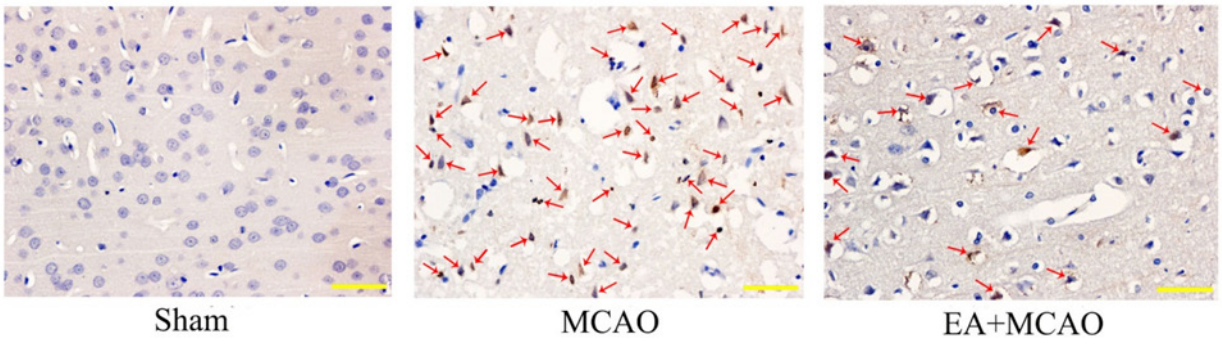

Fig.1 Expression of TUNEL positive cells in each group (10 × 40, bar scale: 50μm)

Note: The red arrows show TUNEL positive cells.

CHINMEDOMICS APPROACH TO EXPLORE THE EARLY INTERVENTION EFFECTS OF SHENG-MAI-SAN ON ALZHEIMER'S DISEASE.

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Abstract In this study, we investigated the early intervention effects of Sheng-mai-san (SMS) against d-gal combined with AlCl3 induced Alzheimer's disease (AD) in SD rats. SMS pretreatment significantly attenuated AD rats learning and memory impairment. Lipid metabolism, Carbohydrate metabolism, Vitamin metabolism and Energy metabolism were all involved in the improvement of SMS on delaying AD development. 20(R)-Ginsenoside Rh1, Schisandrin, Gominsin D, Ginsenoside Rh4, Schisandrol B, Schisantherin B, γ-Schisandrin, Schisandrin B and 6 metabolites from lignans in Schizandra Fruit may be the material base of SMS therapeutic actions. The results demonstrated that SMS exhibited significant preventive effects on AD, multi-components in SMS regulated multi-metabolic pathways may be the related mechanism.

Key words: Alzheimer's disease, Sheng-mai-san, Chinmedomics

Alzheimer's disease (AD) is a common progressive neurodegenerative disease that gradually deprives the patient of cognitive function, ability, language, visualization skills, eventually causes death1. Early intervention may be an effective means of AD prevention. Sheng-mai-san (SMS) is classic Chinese formulae composed of Ginseng (root of Panax ginseng), Ophiopogon Tuber (the enlarged part of the root of Ophiopogon japonicus) and Schizandra Fruit (the fruit of Schisandra chinensis). It has been applied for heart and blood diseases for thousand years in China and recent research showed that SMS possesses cognitive-enhancing activity2. Chinmedomics, defined as "elucidating the therapeutic and synergistic properties and metabolism of Chinese medical formulae and related metabolic pathways using modern analytical techniques" has recently demonstrated significant potential in assessing TCM3. In this study, a chinmedomics approach was applied to investigate the preventive effects and the active ingredients of SMS on AD model rats.

Objective The key metabolic pathways and the pharmacodynamic material base of SMS early intervention on AD were clarified to provide evidences for explanation of multi-components and multi-target synergistic therapeutic mechanism of SMS.

Materials and Methods Based on the d-gal combined with AlCl3 induced aging AD rats model, classic behavior test was first employed to validate the effective intervention of SMS, then Chinmedomics technology platform was introduced to reveal the active constituents in SMS and the influenced metabolic pathways.

Results and Discussion In the orientation navigation test, rats in SMS group spent less time to reach the platform on the training days compared to the model group ($p < 0.05$). SMS group reduced escape latency compared to the model group during the test ($p < 0.05$). In the spatial exploration test, rats in SMS group got higher numbers of platform crossings than in the model group ($p < 0.05$). In both of two tests, the model group and the control group were significantly different and the performance of the SMS group was more close to the control group. These results indicated that SMS treatment ameliorated cognitive deficits in AD model rats.

The metabolic profiles of plasma samples were obviously separated between SMS group and AD model group. The related metabolite biomarkers of AD disturbed by SMS interposed were including L-Lysine, Uridine, L-Leucine,

Formylanthranilic acid, Deoxyribose 5-phosphate, Glycoursodeoxycholic acid, 3,4-Dihydroxy-phenylacetaldehyde, 9-OxoODE, Prostaglandin J2, 3-Oxohexadecanoic acid, Arachidonic acid, Tetrahydrodeoxycorticosterone, Cholesterol sulfate, Oleamide, Alpha-Tocotrienol, 3-Hydroxyoctanoic acid and Vitamin D3, these biomarkers were mainly involved in the following pathways: Lipid metabolism, Carbohydrate metabolism, Vitamin metabolism and Energy metabolism.

The constituents absorbed into blood after oral administration of SMS were analysed by PCA and Metabolynx. 25 constituents were evaluated consisting of 12 prototype and 13 metabolites. After importing the information of 25 constituents and potential biomarkers into PCMS software, 14 constituents were found the most associated with potential biomarkers, including 20(R)-Ginsenoside Rh1, Schisandrin, Gominsin D, Ginsenoside Rh4, Schisandrol B, Schisantherin B, γ-Schisandrin, Schisandrin B and 6 metabolites from lignans in Schizandra Fruit, which may be the material base of SMS therapeutic actions.

This is the first study that reveals SMS improved AD rat cognitive disorders through regulating multiple metabolic pathways and the effective material base were Schisandrin etc, which could be useful to improve the therapeutic regimen of SMS for the interventional treatment of the early stage of AD.

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THE RESEARCH OF RADIX REHMANNIAE POLYSACCHARIDE INDUCED TUMOR CELL APOPTOSIS BASED ON AKT SIGNAL PATHWAY

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Objective: Tostudythe effect ofradix rehmanniae polysaccharideonexpression of p53, Akt, B-cell lymphoma-2(Bcl-2), Bcl-2-associated X protein(Bax), cytochrome c (cyt-C) and cysteinyl aspartate specific proteinases (caspase-3) genes in tumor-bearing mice.To study the influence of immune function.

Methods:

1.Immunohistochemical assay to detectthe P53, Cyt-C and Caspase-3 proteins in the mice tumor tissue.

2.Real Time-PCR was used to analyze the expression of Akt, Bcl-2, Bax, cyt-C and caspase-3 mRNA in tumor tissue.

3. Western blot was used to analyze the expression of Akt, Bcl-2, Bax, cyt-C and caspase-3 proteins in tumor tissue.

4. ELISA assay to detect serumTNF-α, IL-2, and IFN-γ content.

Results:

1. The immunohistochemical detection showed that the expression of P53 protein in tumor tissues of mice inthe high-dose radix rehmanniae polysaccharide group is higher than the model control group ($P<0.05$), the mid-dose group was significantly higher than the model control group ($P<0.01$), the positive control group and the combination group were obviously higher than that of model control group ($P<0.001$); to the expression ofCyt-Cthe mid-dose radix rehmanniae polysaccharide group is higher than the model control group ($P<0.05$), the positive control group and the combination group is obviously higher than that of model control group ($P<0.01$), the combination group was obviously higher than that in the positive control group ($P<0.01$); to the expression of Caspase-3 the mid-dose radix rehmanniae polysaccharide group, the positive control group and the combination group was obviously higher than that of in the control group ($P < 0.01$), the combination group was higher than the positive control group ($P<0.05$).

2. Real Time PCR detection showed that the Akt and Bcl-2 mRNA content in the mid-dose group, the positive group and the combination group were lower than that of in the model group. Oppositely, the Bax, Cyt-C and Caspase-3 mRNA content in these groups were higher than that of the model group.

3. Western blot analysis found that the Akt and Bcl-2 proteins in the mid-dose group, the positive group and the combination group were lower than that of in the model group. Oppositely, the Bax, Cyt-C and Caspase-3 proteins in these groups were higher than that of the model group.

4.ELISA examination found that compared with the model group, the TNF-α in serum was higher($P<0.05$); the IL-2 and IFN-γ was obvious higher($P<0.01$). And the TNF-α, IL-2 and IFN-γ in themid-dose, the high-dose radix rehmanniae polysaccharide group and the combination group were obviously higher than that of in the model group. Compared with the positive group, the TNF-α in the combination group was obviously higher($P<0.01$), the IFN-γ is higher($P<0.05$).

Conclusion: