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.

References

1. Eliezer Masliah. Neuropathology: Alzheimer's in real time [J]. Nature, 2008, 451, 638-639.

2. Giridharan VV, Thandavarayan RA, Konishi T. Effect of Shengmai-san on cognitive performance and cerebral oxidative damage in BALB/c mice [J]. J Med Food. 2011, 14(6): 601-9.

3. Xijun Wang, Aihua Zhang, Hui Sun. Future Perspectives of Chinese Medical Formulae: Chinmedomics as an Effector [J]. OMICS. 2012, 16(7-8): 414-421.

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

Ying Liang Ji Li

(Heilongjiang University of Chinese Medicine, Heilongjiang Harbin 150040) iangying825@163.com 18904888889

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:

1. Radix rehmanniae polysaccharidecan promote the tumor suppressor gene p53 expression.

2. Radix rehmanniae polysaccharidecan induce tumor apoptosis through inhibiting the Akt and Bcl-2 genes expression, increasing the Bax gene expression.

3. Radix rehmanniae polysaccharide can start the mitochondria inducing apoptosis pathway through promoting the Cyt-C and Caspase-3genes expression.

4. Radix rehmanniae polysaccharide can enhance the mice immune function by promoting the cytokine TNF-α, IL-2 and IFN-γ expression.

5. Radix rehmanniae polysaccharide can enhance the anti-tumor effect of cyclophosphamide.

Key words: radix rehmanniae polysaccharide; Akt; Bcl-2; Bax; Cyt-C; Caspase-3

FRAGMENTATION PATTERN OF SPIROSTANOL STEROIDAL SAPONINS FROM ANEMARRHENA ASPHODELOIDES

Yong-Gang Xia, Feng-Qiu Gong, Xin-Dong Guo, Jun Liang, Hai-Xue Kuang

Heilongjiang University of Chinese Medicine, Heilongjiang, China

Abstract: Anemarrhena asphodeloides (A. asphodeloides) rhizome roots of liliaceous is widely used to clearing heat-fire, nourishing Yin and moistening dryness, and recorded in “Chinese pharmacopoeia” 2015 edition [1]. So far, more than 50 steroid saponins have been isolated from A. asphodeloides, which is widely used to decrease blood glucose levels, inhibit platelet activity and carcinoma activity [2-4]. This study applying UPLC-MS/MS methods to analyze Anemarrhena spirostanol reference standards. Finally, fragmentation regularities of four spirostanol steroid saponins are summarized. The four reference standards (RSs) are timosaponin AⅡ (1), timosaponin AⅢ (2), dioscin (3), ophiopogonin D’ (4) and gracillin (5).

Key words: Anemarrhena asphodeloides; spirostanol steroidal saponins; UPLC-MS/MS

Objective: To analysis the fragmentation pattern of spirostanol steroidal saponins from Anemarrhena asphodeloides with UPLC-MS/MS.

Materials and methods: Reference standards were purchased from Chengdu Must Bio-technology Co., which the purity of each standard compound was determined to be over 97% by normalization of the peak areas detected by LC-MS analysis. Separation was achieved on Waters UPLC HSS T3 column (2.1 mm ×150 mm, 1.8 μm) and HSS T3 guard column (2.1 mm × 5 mm, 1.8 μm), in the experiment. A contained 0.6308g/L ammonium formate in water and B was acetonitrile; both of them contained 0.1% formic acid. Sciex Qtrap-4000 detector, [M+NH4]+ data were provided in the positive mode. MIM-IDA-EPI mode were used to scan RSs. In MIM transitions, (Q1) and (Q3) were both [M+NH4]+. DP values were set 50 eV and CE values were set 5 eV. Ion spray voltage was set at +5500 V, interface heater was on and turbo spray temperature was 400 ℃. Both nebulizer gas (gas 1) and heater gas (gas 2) were set at 50 psi. EPI scan was performed at a scan rate of 4000 Da/s. The CE of EPI was set at 8, 15 and 35 eV, respectively.

Results: Similar deductive reasoning was applied to spirostane-type RSs 1-5. Two concise and clear [M+NH4]+ and [M+H]+ ions were obviously observed in the ESI+-EPI (CE,8) spectra for RSs 1-5. In the ESI+-EPI (CE,15) spectra, successive losses of sugar moieties one-by-one were also generated to provide [Agly.+H]+ (RSs 1-5). In addition, the fragmentations of aglycone ions were readily observed in the ESI+-EPI (CE, 35) spectra. Two corresponding ion transitions were involved in [S4+H]+→[S4-C8H16O2+H]+→[S4-H2O-C8H16O2+H]+ (Δm= 144 and 18 Da) and [S4+H]+→[S4-H2O+H]+→[S4-H2O-C8H16O2+H]+ (Δm = 18 and 144 Da) for RSs 1-5. This could be explained through the elimination of E rings and water molecules. The major MS/MS fragment pathways of spirostane-type RSs 1-3 were summarized.

Discussion Whatever spirostanol SSs, all were characterized by preferential loss of a NH3 (17 Da) from an ammoniated precursor ion in the ESI+-EPI (CE, 8) spectra and then C-3 sugar moiety to afford corresponding protonated aglycones in the ESI+-EPI (CE, 15) spectra. As expected in ESI+-EPI (CE, 35) spectra, diagnostic loss of 144 Da from protonated spirostane-type aglycones was attributed to the absence of an oxygen substitution at the F ring while a neutral loss of 160 Da from protonated aglycones could be ascribed to the presence of an OH group (often seen at C-23) in the F ring. The foregoing deduction processes repeated, neutral losses of 142 and 158 Da ions were readily inferred from corresponding protonated spirostanol aglycones for A. asphodeloides SSs. The neutral loss of 142 Da may be explained by the presence of an additional double bond in the F ring (often seen at Δ25(27)-), while a diagnostic loss of 158 Da could be interpreted by the presence of both a double bond (often seen at Δ25(27)-) and an OH group in the F ring (often seen at C-23).

References:

[1] Xia Lei, Jie Zhang, Yuan Li, Qiu-Hong Wang, Xiao-Lin Su, Chang-fu Wang, Yong-Gang Xia, Hai-Xue Kuang. Exploring effective components of laxative effect of Anemarrhena Rhizome based on Chinese herbal processing theory. China journal of Chinese Materia Medica. 2015;40 (7):1283-1286.

[2] Ying-yi Wang, Bao-lin Guo, Li-jun Zhang. Pharmacological functions of chemicals in rhizoma anemarrhenae. Science & Technology Review. 2010;28 (12):110-115.

[3] Xin-Huan Sun, Yu-Wei Zhang, Xue-Feng Huang. Research Progress of Chemical Constituents and Pharmacological Effects of Anemarrhena Asphodeloides. Strait Pharmaceutical Journal.2015;27(3):6-12.