Application of Immuno-Printing Method in the Identification of Astragaloside IV in the Active Ingredients of Traditional Chinese Medicine

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Abstract: Astragalus membranaceus is the most commonly used material in traditional Chinese medicine. Astragaloside is an important and active ingredient. At present, the value of astragaloside has been paid more and more attention by people with the development of medical technology. Astragalus membranaceus (Astragalus membranaceus) is a dried plant root, which contains many chemical components. However, there are still many areas can be improved for the quality detection technology of astragaloside IV. Immunoblotting is a commonly used method of identification. In this paper, the concepts of immunoblotting and astragaloside IV were introduced firstly. Then the application of immunoblotting in the identification of astragaloside IV and an active ingredient of traditional Chinese medicine were analyzed from three processes: material preparation, preparation of drugs and instruments, preparation of astragaloside IV solution and identification of astragaloside IV. Finally, the application of polyacrylamide electrophoresis is helpful to reduce experimental errors and make the results more accurately. Immunoblotting assay has high feasibility for the identification of astragaloside.

1. Research Background

1.1 Literature review

Ding Hetian, Zhu Lihua, and Zhang Qingbo used cell counting plates to measure cell proliferation activity. After the experiment, it was found that astragaloside IV inhibited immune function (Ding et al., 2014). Western blotting has gradually become a popular technique in modern biological experiments. In fact, in the practical application of this method, if we do not improve the method, only use the traditional and conventional immunoblotting method, it will lead to some unavoidable problems, such as the waste of antibodies in the process of using this method. So through exploring, a new method of saving antibody was summarized. The rats in the experiment were randomly divided into two groups. After the retinal tissue was taken alive, homogenate was carried out, and the total amount of different proteins was taken in the Meizu experiment to improve the experiment. After improving the immunoblotting method, the aim of saving antibodies was achieved (Shang et al., 2012). Yang Jingyuan, Wang Lihong and others have found that astragaloside IV can inhibit the proliferation of SPC-A-1 cells and increase the apoptotic rate (Yang et al., 2012). Ningwei et al.. found that astragaloside has protective effect on proteins in the process of heavy metal destructive protein expression (Ning et al., 2012).

1.2 Purpose of research

Astragaloside IV is the most commonly used index to evaluate the quality of Astragalus membranaceus. The development of modern medicine has promoted the in-depth study of traditional Chinese medicine. Astragaloside A, the main component of Astragalus membranaceus, can reduce blood sugar and anti-aging, and is also helpful to immune regulation, protection of small vessels and other organs, anti-virus and other functions. At present, many enterprises have found the prospects of new drug development of astragaloside A. The commonly used method for determination of astragaloside IV in Astragalus membranaceus is HPLC-ELSD. However, this method is time-consuming and tedious in sample processing, and is affected by many other factors.

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However, this method will lead to a low recovery rate of the target sample and produce some qualified edge detection samples. There are some literature reports about the inaccuracy of the treatment methods. In some experiments, sample pretreatment is simplified to ultrasonic treatment, but the results are still unsatisfactory. Therefore, in order to establish a new method for the identification of astragaloside, we should improve and simplify the treatment method of astragaloside samples. In order to carry out the experiment quickly and simply, the experimental data with high accuracy and stability can also be obtained. This paper is based on this background, using polyethersulfone (PES) membrane as carrier, established the immunoblotting method. This method can be used for rapid identification and quantitative analysis of astragaloside. Therefore, it is of great significance to study the application of immunoblotting in the identification of astragaloside.

2. Overview of related theories

2.1 Summary of the Basic Theory of Immunoblotting

Immunoblotting was first proposed in the early 80s of this century. This method combines gel electrophoresis and biomolecular affinity technology, and is widely applied in the detection of proteins. It is highly specific and highly sensitive. This technology first needs to isolate protein samples, which need to be separated by polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane or solid phase carrier such as polyvinylidene fluoride two membrane. Solid phase carriers can ensure that polypeptides separated by electrophoresis retain their original biological activity, and the form of protein adsorption is non-covalent bond. This technique of protein identification has been widely used in immunogenetics and biochemistry. With the continuous development, it has gradually become an important research tool for identification and detection of chemical constituents (Jang and Zhang, 2016).

2.2 A Summary of the Basic Theory of Astragaloside IV

Astragalus membranaceus is the dry root of Astragalus membranaceus and Astragalus mongolica. Astragalus membranaceus contains many active ingredients such as polysaccharides and saponins which are useful for life. Many minerals in Astragalus membranaceus have been separated and identified. Saponins, as the active ingredients of this traditional Chinese medicine, have been detected and identified, and more than ten kinds have been separated. Astragaloside A, an active component of Astragalus membranaceus, is an important index for judging the quality of Astragalus membranaceus. Separation and identification methods have been paid more and more attention by professionals. Identification methods are also in the process of continuous development and improvement. The content of astragaloside A is generally between 5% O and 18% o, and there are more varieties of astragaloside in the range of 10 ~15 (Wei et al., 2017). In addition to the identification methods of astragaloside, the basic characteristics and important role of astragaloside have also become an important part of the general concern of the community. Astragaloside A has a great influence on the development of cardiovascular system, but also has a protective effect on cardiovascular system. It can up-regulate the expression of genes related to blood vessel and myocardium, effectively scavenge oxygen free radicals, and improve the elasticity of vascular inner wall and the activity of myocardial cells. For neurodegenerative diseases such as Parkinson's disease and stroke, astragaloside can repair neuronal damage and loss (Wang et al., 2018).

3. Application of immunoblotting in the identification of active ingredient of Radix Astragali

3.1 Materials and drugs and instruments

Astragaloside IV, Nanjing Chunqiu Biological Engineering Co., Ltd. The water is purified water, acetonitrile is chromatographically pure, and other reagents are of analytical grade. Methanol, Shanxi Jinfeng Coal Chemical Co., Ltd. N-butanol, Nanyang Jinghong New Energy Technology Development Co., Ltd. Ammonia test solution, Shanghai Chemical Laboratory Equipment Co., Ltd.

D101 type macroporous adsorption resin column, Beijing Huakeyi Technology Co., Ltd. Ethanol, Weifang Mingyang Experimental Analytical Instrument Co., Ltd. DYCZ-24DN electrophoresis instrument, Beijing Haitian Youcheng Technology Co., Ltd. Low temperature high speed centrifuge, Shanghai Gaozhi Precision Instrument Co., Ltd. Enhanced ECL Chemiluminescence Detection Kit (ready-to-use), Nanjing Nuoweizan Biotechnology Co., Ltd. HELIX MC Plus Rare Gas Mass Spectrometer, Thermo Fisher Scientific (China) Co., Ltd. FD-CNMR-I nuclear magnetic resonance instrument, Shanghai Tianwei Teaching Experimental Equipment Co., Ltd. YP200KN-5 electronic balance, Shanghai Mi Qingke Industrial Co., Ltd. Constant temperature water bath, Weifang Mingyang Experimental Analytical Instrument Co., Ltd. Chromatography workstation, Beijing Li Desheng Innovation Technology Co., Ltd. Ultrasonic cleaner, Dongguan Hongshun Automation Equipment Co., Ltd. Soxhlet extractor, Wuxi Woxin Instrument Manufacturing Co., Ltd.

3.2 Method

3.2.1 Preparation of astragaloside IV solution

Weigh 4g of astragaloside IV with an electronic balance and accurately weigh it. The weighed 4 g of the drug was placed in a Soxhlet extractor and methanol was added to the Soxhlet extractor in a volume of 40 ml of methanol. The drug was placed in a Soxhlet extractor overnight, and an appropriate amount of ethanol was added again, and the drug was heated and refluxed for 4 hours. After the extract was heated to reflux, the solvent was recovered and the solute was concentrated to dryness. 10 ml of distilled water was added to the residue, and the residue was dissolved by heating and extracted with n-butanol for four times with water, and shaken each time with shaking. The water-saturated n-butanol and the residue solution were each made up to 40 ml. Thereafter, it was washed twice with an ammonia test solution, and the volume was adjusted to 40 ml each time. The ammonia solution was poured out, the water-saturated n-butanol was evaporated to dryness, and the residue was again dissolved in distilled water, and placed in a cold state, and passed through a D101 type macroporous adsorption resin column. The parameters of the D101 type macroporous adsorption resin column are an inner diameter of 37.5 px and a column height of 300 px. After passing through a D101 type macroporous adsorption resin column, the residual liquid was eluted with 50 ml of distilled water, and the aqueous solution was poured off. The elution was continued with 30 ml of 40% ethanol, the eluate was discarded, and eluted with 80 ml of 70% ethanol, and the final eluate was collected and evaporated to dryness. The residue obtained by the treatment was dissolved in a methanol solution, poured into a 5 ml volumetric flask, and made up to volume with methanol, and the resulting solution was shaken to obtain a solution of astragaloside IV.

3.2.2 Identification of Astragaloside IV, an active ingredient of traditional Chinese medicine by immunoblotting

In this paper, the biological activity of astragaloside IV was identified by anti-serum immunoblot analysis of cells carrying Epstein-Barr virus in mice. Since cells carrying Epstein-Barr virus carry the early genome of this virus, cell antiserum carrying Epstein-Barr virus can detect polypeptides caused by tissue polypeptide antigens, which has been confirmed by previous studies. The cells carrying the Epstein-Barr virus were cultured in a medium in an environment of 5% CO 2 and 37 ° C. The medium consisted of 20 mmol/L HEPES and 10% bovine serum Dulbecco's phosphate buffer. In each medium, the cell content was 2 x 105 cells/ml. The cells, the separated components, and the carcinogen were cultured together in 20 ml of the medium for 2 days (48 h). The carcinogen contained 65 nmol/L tissue polypeptide antigen and 1 mmol/L sodium n-butyrate. With or without the addition of a carcinogen, the cells were lysed with a buffered extraction solution containing 1% polyoxyethylene (20) sorbitan monolaurate, 1 mmol/L ethylenediaminetetraacetic acid and 1% sodium deoxycholate, in the final extract. The cell content is approximately 106 cells/25L. The protein content in the cell extract was approximately 20 g, and the extract was subjected to electrophoresis on a 10% polyacrylamide (PAM) lamellar gel containing 0.1% sodium lauryl sulfate. The protein in the cell extract is subjected to electrophoresis and then transferred from the solution to the nitrocellulose membrane. Finally, immunostaining was performed with a 1:50 dilution of EB

virus cell antiserum, a 1:500 dilution of biological antibody, and a 1:500 dilution of mycobacterin. The cell viability of the extracted components was identified using a trypan blue exclusion assay to determine the number of viable cells.

3.3 Identification of Astragaloside IV, an active ingredient of traditional Chinese medicine by immunoblotting

Astragaloside IV is a high-purity drug extracted from Astragalus and belongs to the class of saponins. Amino acids are often used in experiments as a quality standard for the evaluation of xanthines. Astragaloside IV plays an important role in immunity and disease resistance, and can enhance immunity and disease resistance. The most important biological activity of baicalin is astragaloside IV, which is baicalin IV. Astragaloside IV has the antiviral effect of the polysaccharide function of Astragalus membranaceus while it has the function of Astragalus polysaccharide. In this study, the biological activity of astragaloside IV was identified by anti-serum immunoblot analysis of EB virus-bearing cells in mice, and the amino acids in astragaloside IV were rapidly assayed. The main results are as follows: (1) Astragaloside IV has an effect on the gene expression profile of rat myocardium. Immunoblotting shows that Astragaloside IV has an effect on the development of cardiovascular and myocardial function. (2) Astragaloside IV can significantly promote the proliferation of neural stem cells in vitro and induce the formation of nerve cells. (3) Astragaloside IV has a regulatory effect on the immune function of the body, and can increase the production of lymphocytes and antibodies in vitro and in vivo. The results showed that the Western blotting method is feasible for the identification of the active ingredient of Chinese traditional medicine. Compared with high performance liquid chromatography and enzyme-labeled immunosorbent assays, immunoblotting is simpler and more convenient, and does not require huge cost and large drug consumption. The immunoblotting method provides good technical support for the development and utilization of medicinal plant resources and the rapid analysis and identification of the activity of traditional Chinese medicine ingredients.

4. Conclusion

There are many steps in the immunoblotting process, and the operation is complicated, so the probability of error in the experiment is high. Polyacrylamide gel electrophoresis has been widely used in biochemical experiments, especially in immunology. This method is beneficial to reduce experimental errors and make the results more accurate. Through the study, it is known that the immunoblotting method is feasible for the identification of the active ingredient of traditional Chinese medicine, astragaloside. And astragaloside has an effect on the development of cardiovascular and myocardial function; astragaloside promotes the development of the nervous system; astragaloside IV has a regulatory effect on the immune function of the body. However, the toxicological effects of astragaloside IV have not been studied. In view of the fact that astragaloside IV is the most advantageous component of the biological activity of xanthine, it is recommended to develop a more comprehensive identification method to ensure the safety of astragaloside IV. To provide practical and useful reference materials for drug research and development.

References

[1] Ding H.T., Zhu L.H., Zhang Q.B., et al. (2014). In Vitro Experimental Study on the Effect of Astragaloside on Immune Function, Chinese Journal of Coal Industry Medicine, 17 (12), 21-24.

[2] Shang L., Huang K., Cao Y. Q., et al. (2012). An Improved Immunoprinting Method, Modern Biomedical Progress, 12 (32), 68-70.

[3] Yang J. Y., Wang L. H., Wang L. (2016). The Effect and Mechanism of sPC-A-1 Cell Proliferation in Human Lung Cancer, Proprietary Chinese Medicines, 38 (8), 18-20.

[4] Ning W., Liao X. G., Wang Y., et al. (2014). The Damage of Cadmium to the Blood Testicular Barrier of Rats and the Protective Effect of Jaundice Methylene, Anatomy Journal, 45 (5), 704-709.

[5] Jiang H. G., Zhang J. G. (2016). Jasphine Inhibits the Expression of Myocardial Fibrosis and Conversion Growth Factor Beta 1 in Rats with Chronic Heart Failure, New Medicine, 47 (1), 27-33.

[6] Wei B., Jiang X. J., Lu B. et al. (2017). Effect of Ox-LDL on the Expression of Endothelial Cell CD40 Signal and Anti-inflammatory Effect of Jaundice, Journal of Tianjin University of Traditional Chinese Medicine, 36 (4), 82-85.

[7] Wang L. L., Feng L. J., Liu J. F. (2018). The Protective Effect and Mechanism of Jaundice on the Oxidative Stress Damage of Human Glomerular Membrane Cells in High-sugar Environment, Chinese Journal of Clinical Pharmacology, 34 (9), 79-82.