Studies on the Pharmacological Applications of Recombinant Human Serum Albumin

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Abstract: As one of the most important proteins in plasma, human serum albumin (HSA) has multiple physiological functions, such as maintaining osmotic pressure, transporting endogenous and exogenous substances, and immunomodulation. However, with the increasing clinical use and relative shortage of plasma raw materials, the shortage of supply and price of HSA have gradually become more and more obvious. Recombinant Human Serum Albumin (rHSA) has received extensive attention in the field of pharmacy due to its advantages of avoiding blood contamination, controllable production capacity, and high quality consistency. The rHSA expressed by genetic engineering technology has already emerged in industrial production, clinical research, drug carriers and other aspects, and many domestic and foreign companies are laying the groundwork for the industrialization of related products. In this study, the structure and function of recombinant human serum albumin, preparation technology, clinical application prospect, quality control and industrialization process are systematically described with reference to the literature and practical progress in recent years, and the future development direction of the recombinant human serum albumin is prospected.

Keywords: Recombinant human serum albumin; Pharmacological applications; Plasma

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I. Introduction

Human Serum Albumin (HSA) is one of the most widely used blood products in clinical practice, which is widely used in the treatment of hypoalbuminemia, cirrhosis ascites, severe burns, surgical wounds and shock caused by major diseases. At the same time, human serum albumin can also be used as stabilizer or excipient for the production and preservation of vaccines, monoclonal antibody drugs and various types of recombinant protein drugs. Due to the scarcity of plasma resources and human plasma collection of regional, seasonal, epidemic impact and other factors, the annual production of human serum albumin is far from meeting market demand, import dependence is always high. In order to break through the bottleneck of plasma source, since the 1980s, people have been exploring the feasible path of using biotechnology to prepare recombinant human serum albumin. After decades of research, recombinant human serum albumin (rHSA) has been expressed in different systems, such as yeast, fungi, and transgenic plants, and has gradually entered the clinical trial stage.

In recent years, with the rapid progress of genetic engineering and bioprocess technology, rHSA has made great progress in industrial scale and quality stability, and a number of internationally competitive enterprises have emerged. Recombinant human serum albumin has significant advantages in overcoming the risk of blood-borne disease transmission and guaranteeing yield and quality consistency, and has gradually become one of the key research areas in the pharmaceutical industry [1]. At present, a number of domestic and foreign companies have completed or are conducting phase I, phase II and even phase III clinical trials of rHSA in anticipation of early approval for marketing, with a view to further meeting clinical needs. In this paper, we will systematically describe

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the basic principles, pharmacological properties, clinical application potential and future development trend of recombinant human serum albumin in the light of the latest research literature and industrial practice.

2. Structure and Function of Recombinant Human Serum Albumin

(1) Structural overview

Human serum albumin is a single-chain protein consisting of 585 amino acids with a molecular weight of about 66.5 kD and an isoelectric point between 4.7 and 4.9. Its spatial conformation is relatively stable, presenting an approximate heart or ellipsoid shape. Since HSA itself is not modified by glycosylation, protein folding mainly depends on its own amino acid sequence and disulfide bonds. In the blood circulation, the half-life of HSA is about 17-19 days, and it synthesizes about 10-15g per day, with a relatively stable endogenous synthesis rate. The high concentration and stable three-dimensional structure make it excellent in carrier function.

(2) Main physiological functions

First, HSA is able to maintain plasma colloid osmotic pressure and regulate the balance of the internal environment of body fluids, which is its most common use in clinical emergencies. Second, HSA has an important transport and binding ability, and can carry a variety of endogenous small molecules (e.g., hormones, bilirubin, free fatty acids, etc.) and exogenous drugs (e.g., certain chemotherapeutic drugs or small molecule inhibitors), effectively prolonging the drug half-life [2]. Thirdly, HSA is also involved in immune regulation, and is able to bind to certain toxic substances and play a detoxifying and buffering role to a certain extent. It is based on these unique properties that HSA is indispensable in clinical applications.

(3) Comparison of recombinant human serum albumin with natural proteins

In terms of amino acid sequence and overall tertiary structure, rHSA is highly consistent with HSA, and this consistency provides an important molecular basis for its equivalence in function and application. Using modern biotechnology and purification processes, the protein purity, stability and other key quality indicators of rHSA have reached a level comparable to that of plasma-extracted HSA. Studies have shown that rHSA exhibits highly similar functional properties to natural HSA in its ability to maintain colloid osmotic pressure, transport small molecules, and bind endogenous and exogenous compounds, which lays the foundation for its potential in clinical alternative therapies. In recent years, several clinical trials surrounding rHSA have demonstrated that rHSA performs close to ideally with natural HSA in terms of both pharmacokinetic and pharmacodynamic parameters, e.g., there are almost no significant differences between the two in terms of in vivo distribution, metabolic pathways, and half-life. In addition, the incidence of rHSA-related adverse events was also consistent with that of natural HSA and did not show significant immunogenicity differences. This suggests that the improvement of the recombinant process has effectively addressed the issues of host residual impurities and potential immune risks, resulting in a high clinical acceptability of rHSA in terms of safety and efficacy. More importantly, the production of rHSA is free from the dependence on plasma resources while circumventing the potential threat of blood-borne disease transmission, providing a more stable and reliable source of supply for clinical applications. This technological advancement not only represents a major breakthrough in the biopharmaceutical industry, but also opens up new avenues for human health management.

3. Preparation Process and Progress of Recombinant Human Serum Albumin

(1) Choice of expression system

Since the first successful expression of the HSA gene in E. coli in 1981, there have been continuous attempts

to obtain higher yields of active proteins in various host systems. Currently, there are four major expression systems: (1) prokaryotic systems, such as escherichia coli; (2) yeast systems, such as Brettanomyces cerevisiae and Saccharomyces cerevisiae; (3) plant systems, such as transgenic plants, such as rice, tobacco, etc.; and (4) mammalian cells or transgenic animals, etc. Each system has different advantages and disadvantages. Each system has different advantages and disadvantages: the prokaryotic system has a high expression capacity but is difficult to fold, the yeast system has a large production capacity and a controllable cost, the plant system is easy to scale up and has the advantage of safety, and the mammalian system facilitates correct folding but is expensive. At present, many domestic and foreign manufacturers are focusing on the Picros yeast or rice endosperm system, aiming to obtain high quality rHSA stably.

(2) Purification and quality control

Due to the good water solubility and stability of HSA under physiological conditions, impurity proteins can be removed relatively efficiently by chromatography and ultrafiltration if environmental factors can be effectively controlled during the reconstitution process. However, the yeast system usually secretes some protein hydrolases and polysaccharides, and the plant system contains chlorophyll, phytotoxins and other potential impurities, which need to be separated and purified through a series of processes such as centrifugation, flocculation, ultrafiltration, ion exchange, hydrophobic chromatography and so on. The purity of the final rHSA must be more than 99%, and the endotoxin must be lower than the national pharmacopoeia to ensure the safety and effectiveness of the preparation.

(3) Domestic research and industrial deployment

In recent years, a number of domestic companies have made breakthroughs in the clinical trials of rHSA, such as the recombinant human albumin expressed by Pichia yeast in Tonghua Arrowhead Biopharmaceuticals has completed Phase III clinical trials and has been approved for marketing in some countries; rHSA expressed by Wuhan Hoyuan Bio-technology using rice endosperm has entered into Phase III clinical studies, and is actively seeking for industrial cooperation on a global scale. Meanwhile, other companies are further optimizing their production processes and expression systems, trying to expand their scale and connecting to the international drug regulatory system.

4. Current Status of Clinical Application and Pharmacological Research

(1) Replacement of human albumin in the treatment of hypoproteinemia

The most direct clinical application of current rHSA with human plasma-derived HSA is for various types of conditions that result in low blood albumin, such as chronic cirrhosis with ascites, extensive burns, surgical blood loss, infectious shock, and other conditions. Numerous phase I, II and III clinical trials have shown that rHSA does not differ significantly from plasma-derived HSA in terms of recovery of serum albumin concentration, symptomatic improvement, and quality of life enhancement in patients, and its safety profile is in good agreement. The results of trials in patients with cirrhotic ascites are particularly impressive, with positive results in terms of increased plasma colloid osmolality, reduced ascites, and reduced body weight.

(2) Drug carriers and excipients

In addition to its direct use as a therapeutic protein, rHSA is also promising in drug carriers and biologics excipients. Due to its good affinity and biocompatibility, rHSA can be combined with a variety of drugs or active factors to delay their degradation, prolong the half-life in vivo, and enhance drug efficacy. For example, certain antitumor drugs can be combined with rHSA to form nanoscale albumin microspheres or albumin conjugates, which can achieve more effective localization and delivery under the conditions of high protein receptor expression

in cancer cells. In addition, rHSA can be used as a formulation stabilizer for biologics such as vaccines or monoclonal antibodies to help maintain their activity and structural integrity.

(3) Other potential areas of application

With the advancement of clinical needs and technology, the potential of rHSA in tissue engineering, bioartificial liver support systems, and transmembrane protein fusion expression has been gradually emphasized. Albumin is often added to some in vitro cell or tissue culture media to provide nutrients and a stable environment, and by using recombinant products, blood-borne risks and batch differences can be effectively avoided. In terms of bioartificial liver, co-culturing rHSA with hepatocytes to mimic the human liver microenvironment and provide short-term support for liver failure patients is also a promising direction.

5. Major Challenges Facing Recombinant Human Serum Albumin

(1) Cost of production and scaling up

Although rHSA does not require plasma raw material, it relies on advanced fermentation or GM planting equipment, refined purification process and strict GMP workshop environment in the production process, which makes the upfront investment expensive. Especially for large-scale fermentation such as transgenic rice and yeast systems, it is also necessary to build supporting gene operation platforms, downstream purification modules, etc., with high capital pressure and long construction period. If it is not possible to reduce the unit cost in large-scale production, the price of finished products may still be too high, affecting the popularity of clinical applications.

(2) Quality and safety regulation

The clinical evaluation of rHSA by regulatory authorities is particularly stringent due to the historical experience in the use of plasma products with more established pharmacopeial standards. In addition to protein purity, structural characterization, immunogenicity, etc., the potential effects on the human body of residual impurities, protein modifications and folding differences brought by the expression host need to be examined. How to establish a quality evaluation system in line with international standards, improve testing standards and accumulate a long-term safety database is the key to the global success of rHSA.

(3) Marketing and clinical awareness

In the past, clinicians and patients generally accepted plasma-derived HSAs, and often had doubts about new recombinant products, including whether the efficacy is consistent and whether rare adverse reactions will occur. Therefore, companies need to use sufficient clinical trial data and scientific publicity to gradually build up the confidence of doctors and patients in the safety and efficacy of rHSA, and at the same time accelerate the coverage of health insurance policies or commercial insurance in order to benefit more patients.

6. Future Outlook and Development Trends

(1) Diversified expression system and process innovation

With the increasing sophistication of genetic engineering and biosynthesis technology, the expression system of rHSA presents a more diversified and specialized development. This not only brings considerable improvement in production efficiency, but also provides brand new ideas for industrial cost control. Taking the plant system as an example, utilizing rice endosperm or other highly expressed plant organs as the production platform, fully exploiting the advantages of agricultural resources, which can both expand the production capacity and significantly reduce the unit cost of industrial preparation, has become one of the very attractive solutions in the future. Meanwhile,

for the yeast system, the scientific community is actively seeking to optimize its protein expression pathway at the molecular level, for example, to cut down the accumulation of by-products of protein hydrolysis enzymes, so as to enhance the expression amount and reduce the complexity of the subsequent separation process. In addition, in the field of purification processes, the optimization of chromatographic packing, the improvement of impurity removal processes, and the application of highly efficient automated equipment are further promoting the overall efficiency of the industrial chain [3]. With scientists' in-depth understanding of gene regulatory networks and molecular folding mechanisms, it is expected to create more intelligent and refined process platforms in the future, so as to build an integrated and efficient process covering from the selection of expression systems to the development of end-use preparations.

(2) RhSA derivatives and the potential for functionalized design

Continuous advances in modern pharmacology have provided a wide scope for the development of rHSA derivatives. The boundaries of their clinical applications can be further broadened through targeted modification of the amino acid sequence or introduction of functional fragments into the protein structure. For example, fusion of HSA with certain transmembrane segments or specific receptor-binding regions can enhance its targeted delivery ability, a property that is particularly promising in the development of antitumor drugs. Meanwhile, researchers are also trying to extend the duration of drug action in vivo by binding to antibody segments to achieve higher therapeutic efficiency. In drug delivery systems, the unique nanoparticle properties exhibited by rHSA are being focused on, and scientists expect to create safer and more efficient drug carriers through its stability and low immunogenicity advantages. In addition, the potential of rHSA in the fields of tissue engineering, bioartificial organs, and cell culture is also attracting attention. These new directions not only inject more vitality into the traditional albumin research, but also signify that rHSA will carry more missions in the future biomedical field.

(3) Development of globalization and deepening of market competition

The internationalization of recombinant human serum albumin (rHSA) is gradually accelerating against the backdrop of expanding global market demand. Emerging markets, represented by the Asia-Pacific region, are showing rapid growth in demand for rHSA, while mature markets such as Europe and the United States have set up technical barriers for the global supply chain by demanding high standards of efficacy and safety. If the leading domestic enterprises can actively participate in international multi-center clinical studies and obtain market access in key regions, they will enhance their international competitiveness and strive for a greater voice for China's biopharmaceutical industry. At the same time, with the expiration of recombinant albumin patents, the differentiated competition within the industry may further intensify in the future. Technology licensing, international mergers and acquisitions, and cross-border cooperation will be more widely used to promote resource integration and complementary advantages among enterprises. In addition, enterprises need to focus on building production and quality management systems in line with international standards and breaking down regulatory barriers, so as to develop a broader global market for their products.

7. Conclusion

The emergence of recombinant human serum albumin (rHSA) has provided a brand new solution idea to alleviate the insufficient supply of human serum albumin, reduce the risk of transmission of blood-borne diseases as well as enhance the application value of rHSA in the field of pharmacy. After decades of research and development exploration, the current rHSA has taken key steps in yeast and plant systems, and a number of companies have entered phase III clinical trials and even been approved for marketing and sales in some regions, marking that recombinant human serum albumin is gradually moving towards maturity. From a pharmacological point of view, rHSA is already highly similar to plasma-derived albumin in terms of structure, function and safety, and is expected

to achieve breakthroughs in cost and accessibility to meet broad clinical and industrial needs.

However, the full realization of rHSA as a substitute for plasma-derived albumin still requires coordinated development in various aspects, such as large-scale production, cost control, quality evaluation, and marketing. With the aging of the population and the threat of emerging infectious diseases worldwide, the clinical demand for safe, effective, and sustainable albumin products will grow further. At the policy level, it is necessary to provide better regulatory guidance and financial support to relevant enterprises and research organizations; in the academic community, more in-depth research is needed to reveal the potential application scenarios of rHSA and explore its mechanism. It is believed that under the joint efforts of all parties, the industrialization of recombinant human serum albumin will continue to accelerate, making new and greater contributions to the protection of human health.

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