Analysis of Pharmaceutical Processes and Related Problems Using Biotechnology: Taking EPO Production as an Example

Xuyuan Zhang

Department of Life Sciences and Biopharmaceuticals, Shenyang Pharmaceutical University, Shenyang, 11000, China

* Corresponding Author Email: 1910821115@mail.sit.edu.cn

Abstract. Biotechnology is increasingly being used in pharmaceutical production. Solve the problems encountered in the current pharmaceutical process to help more efficient production. It is now possible to produce protein drugs such as EPO with relative maturity. People can use related biotechnology to solve some of the problems encountered in production. However, many biotechnology applications have not yet been integrated into the pharmaceutical process. This study first introduced the role of EPO in human body, the mechanism of EPO and the application field of EPO. Then, the production process and biotechnology involved in EPO generic drugs are analyzed. Finally, the existing shortcomings of relevant processes, processes, technologies and optimization methods are analyzed, and a production model suitable for most protein drugs is obtained. This study provides reference suggestions for the production process and technical optimization of protein drugs for future research, but does not analyze drugs other than protein drugs. Future research can focus on the pharmaceutical process of nucleic acid drugs, polysaccharide drugs and other types of drugs and related problems analysis, and can also supplement and optimize the results of this research.

Keywords: EPO; biotechnology; process; protein drugs.

1. Introduction

Shi Yigong, a tenured professor at Princeton University, pointed that the 21st century is the century of bioscience. Biotechnology is developing at an astonishing rate, allowing people to control living things to a certain extent, and creating convenience for human beings. Biotechnology has many applications. In agriculture, biotechnology can improve crop yield and quality and play an important role in the development of stress-resistant crops. In industry, biotechnology is used to increase productivity, improve food quality, develop food varieties, and to mass-produce industrial enzymes to produce paper. In medicine, biotechnology has a wider range of applications. Vaccine-active immunization is one of the most effective means to prevent infectious diseases. Within weeks of the emergence of COVID-19 cases, the unknown coronavirus had been resolved in the lab and scientists had generated genetic copies of it to make a vaccine. People are using gene therapy to cure previously incurable diseases. It works by modifying a patient's DNA. Currently, gene therapy has been successfully used to treat diseases such as cancer, cystic fibrosis and immune deficiency. Bacteria are used to make protein-based drugs such as erythropoietin (EPO). It can be seen that biotechnology plays a very important role in the pharmaceutical process. In recent years, with the deepening of the research of biopharmaceutical technology, the field of biopharmaceutical has developed rapidly in the pharmaceutical industry [1]. The pharmaceutical technology of biologics in the production process needs to play the best technological advantages to have a strong development potential and application prospects in the pharmaceutical field, and the optimization and upgrading of the current pharmaceutical technology and process can make the health of the broad masses of people better protected [1].

However, people have not yet integrated as many biotechnology applications into the pharmaceutical process. If these technologies can be applied to actual production, it will greatly improve production efficiency. EPO is an important chemical with a wide range of applications, especially in the pharmaceutical and chemical industries. The production process of EPO refers to the process of converting raw materials into final products. This paper will take the production of

EPO as an example to explain in detail the whole process of pharmaceuticals using biotechnology and analyze the related problems. Based on these analyses, this paper aims to provide feasible recommendations for process optimization in the biopharmaceutical field.

2. EPO Overview

2.1. Biological Function of EPO

EPO is mainly synthesized and secreted by capillary endothelial cells and stromal cells around renal tubules, and a small part of EPO can also be synthesized by liver [2]. Here are the main effects of EPO on the human body. EPO, which circulates primarily in blood plasma, can stimulate the transformation of naive blood cells (called primordial hematopoietic cells) into mature red blood cells in the bone marrow. This process is called erythropoiesis or erythropoiesis. Red blood cells are important cells in the blood whose main function is to transport oxygen to the various tissues and organs of the body. By promoting the production of red blood cells, EPO also indirectly increases hemoglobin levels in the blood. Hemoglobin is a protein in red blood cells that carries oxygen throughout the body. By increasing the number of red blood cells and hemoglobin levels, EPO helps maintain oxygen balance in the body. This is essential for the proper functioning of various tissues and organs in the body. EPO can also affect the lifespan of red blood cells, keeping them in the circulatory system for the appropriate amount of time. This helps to ensure that old red blood cells can be replaced by new ones, maintaining the proper number of red blood cells in the blood. The production of EPO is regulated by oxygen levels. When oxygen levels in the body decrease, such as at high altitudes or when anemia occurs, the kidneys release more EPO to stimulate the production of red blood cells, which increases oxygen transport and supply. In general, EPO plays an important physiological role in maintaining the balance of oxygen supply and demand, regulating the number of red blood cells and maintaining normal physiological functions.

2.2. EPOR Is Involved in the Regulation of Oxygen Levels in the Body

EPO exerts its role in the human body through complex biological mechanisms. The following are the main mechanisms of action of EPO. The production of EPO is regulated by oxygen levels in the body. In particular, cells in the kidneys sense the level of oxygen in the blood. When oxygen levels drop, these cells release more EPO. EPO binds to corresponding receptors in the kidney and other tissues, the main receptor being the EPO receptor on the surface of the precursor cells of red blood cells. This combination triggers a series of signaling pathways.

1) When ischemic stroke occurs, hypoxic ischemia of the brain can cause the activation of hypoxic-inducible factor-1 (HIF-1), resulting in the release of brain-derived EPO, which then activates the erythropoietin receptor (EPOR) in brain nerve cells to form EPO-EPOR [3]. The cell signaling pathway mediated by the combination of EPO and EPOR can not only promote the proliferation and differentiation of red blood cells but also have a protective effect on nerve cell injury caused by cerebral ischemia [3]. The EPOR activates the JAK-STAT signaling pathway, which is a common cell signaling pathway. This process involves activation of kinases and activation of a series of signaling molecules, ultimately leading to activation of the intracellular transcription factor STAT (signal transduction and activation of transcription).

2) Activated STAT enters the nucleus and binds to DNA, prompting transcription of specific genes. The role of the pathogenesis of ischemic stroke is also taken as an example. Studies have found that the expressions of JAK1, JAK2, STAT1 and STAT3 in the JAK-STAT signaling pathway are up-regulated after cerebral ischemia, and phosphorylation activation of STAT1 and STAT3 can reduce the expression of anti-apoptotic genes Bcl-2 and Bcl-x, thus promoting neuronal apoptosis [4,5]. From this point of view, this is unfavorable for the treatment of cerebral ischemia, because the bcl-2 gene has a significant inhibitory effect on apoptosis, but the expression of this gene is reduced, resulting in faster apoptosis of brain neurons. However, the activation of this pathway has a positive effect on the angiogenesis and remodeling of cerebral ischemic diseases [3].

3) These specific genes code for key proteins in red blood cell precursors. These proteins include hemoglobin, enzymes associated with hemoglobin synthesis, and other proteins associated with red blood cell development and function.

Through the process mentioned above, EPO eventually causes the original hematopoietic cells in the bone marrow to differentiate into mature red blood cells. These red blood cells enter the bloodstream, increasing the number of red blood cells in the body, raising hemoglobin levels, and improving the oxygen transport capacity of the blood. In general, EPO works by sensing oxygen levels in the body, activating the JAK-STAT signaling pathway, prompting transcription and translation of related genes, and ultimately stimulating the production of red blood cells. This process is a complex and highly regulated biological response that ensures an appropriate physiological response to the need for oxygen.

2.3. Application of EPO in Medical Field

One of the most widespread areas of application for EPO is the treatment of anemia caused by various causes. This includes renal anemia caused by chronic kidney disease, anemia caused by radiotherapy or chemotherapy in cancer patients, chronic diseases, hereditary anemia, etc. By stimulating the production of red blood cells, EPO can increase hemoglobin levels and improve symptoms and quality of life in patients with anemia. For some patients, especially before and after surgery, anemia may occur. In these cases, doctors may consider using EPO to prevent the need for blood transfusion after surgery. EPO can raise the number of red blood cells to reduce the amount of blood transfusions that may be required after surgery, thus reducing the risk of surgery-related anemia. These patients often develop renal anemia, so EPO is used to help regulate the number of red blood cells in dialysis patients and improve the symptoms of anemia. Recombinant human EPO combined with component blood transfusion has good clinical effects in the treatment of chronic anemia, which can effectively improve the level of Hb, RBC and HCT, reduce the level of inflammatory factors, reduce the average blood transfusion volume, and have fewer adverse reactions, which is conducive to improving the anemia state and promoting the rapid recovery of patients [6].

EPO is also used as a blood enhancer because it improves oxygen delivery and increases endurance. However, its use is strictly regulated in sport for anti-doping purposes, and testing methods exist to detect abuse. It is important to note that the use of EPO must be carried out under the guidance of a doctor, as overuse or incorrect use can cause serious side effects, such as thrombosis, high blood pressure, increased risk of cardiovascular events, etc. Therefore, the doctor will decide whether to use and the dosage to use according to the specific situation and needs of the patient.

3. EPO Production Process

3.1. The Production Process of EPO Generic Drugs and the Biotechnology Involved

3.1.1 Selection of host cells

The first step in producing EPO is to select the appropriate host cells. Host cells are often selected based on their ability to properly fold and translate proteins. Common host cells include Escherichia coli and mammalian cells. Escherichia coli has a clear genetic background, can grow rapidly on cheap medium, is one of the most commonly used chassis cells in metabolic engineering and synthetic biology, and is a relatively ideal host cell choice [7].

3.1.2 EPO gene insertion

The EPO gene is introduced into the selected host cell, transforming it into the cells needed for production. This can be done by using plasmids, usually DNA rings with EPO genes, etc. In mammalian cells, plasmid transfection is usually used to introduce EPO genes into host cells.

3.1.3 Culture of host cells

The culture of E. coli is used here as an example. Prepare an appropriate medium, such as Luria-Bertani (LB) medium. The medium is added to the culture bottle and sterilized automatically or manually. Take a frozen tube containing E. coli strains, dip an appropriate amount of bacterial liquid with a sterile straw, and transfer it to the medium. Take care to avoid the introduction of other bacteria or contaminants. Incubate the bottle in a constant temperature incubator or shaker at the appropriate temperature (usually 37°C) and for the appropriate culture time. The environment in the incubator is usually maintained with proper humidity and oxygen supply. During the culture process, density measurements can be made as needed to understand the growth of bacterial colonies. This can be done by measuring the light density of the culture solution, the number of colonies, or the appearance of the bacterial colony. As needed, the culture solution can be passed continuously to subculture the bacterial population until the number of bacteria required for production is reached. At the time of passage, appropriate amount of bacterial liquid was taken and transferred to a new culture bottle containing fresh medium.

3.1.4 Expression and transcription

The culture of E. coli is used here as an example. Prepare an appropriate medium, such as Luria-Bertani (LB) medium. The medium is added to the culture bottle and sterilized automatically or manually. Take a frozen tube containing E. coli strains, dip an appropriate amount of bacterial liquid with a sterile straw, and transfer it to the medium. Take care to avoid the introduction of other bacteria or contaminants. Incubate the bottle in a constant temperature incubator or shaker at the appropriate temperature (usually 37°C) and for the appropriate culture time. The environment in the incubator is usually maintained with proper humidity and oxygen supply. During the culture process, density measurements can be made as needed to understand the growth of bacterial colonies. This can be done by measuring the light density of the culture solution, the number of colonies, or the appearance of the bacterial colony. As needed, the culture solution can be passed continuously to subculture the bacterial population until the number of bacteria required for production is reached. At the time of passage, appropriate amount of bacterial liquid was taken and transferred to a new culture bottle containing fresh medium.

3.1.5 Protein folding and modification

The EPO protein undergoes a complex folding and modification process within the cell, ensuring that the resulting protein is an active, correctly structured EPO. This includes glycosylation and other post-translational modifications that are essential for the bioactivity and stability of EPO. Attention should also be paid to the monitoring of protein expression, folding and modification to ensure the quality and consistency of the final product.

3.1.6 Harvest and purification

After expression and modification, the production process involves harvesting and purifying the cell culture. This usually involves the use of different separation techniques, such as column chromatography, gel filtration, affinity chromatography, etc., to isolate EPO proteins from cell cultures. The purified EPO protein is eventually prepared into a preparation, such as an injection or a freeze-dried powder. These formulations must comply with the appropriate drug regulations and standards.

In general, the process of producing EPO generic drugs involves multiple biotechnology steps such as genetic engineering, cell culture, protein expression and modification, purification, and preparation of preparations. These steps require a high level of technical skill and quality control to ensure the safety and effectiveness of the end product.

3.2. Existing shortcomings and optimization methods of relevant processes and technologies

3.2.1 Host cell selection

Optimizing host cell lines is a method to improve production efficiency and product quality. E. coli is one of the commonly used host cells, but they may not be able to perform proper protein translation and folding, resulting in less active proteins. The use of mammalian cells such as Chinese Hamster Ovary (CHO) cells can improve protein folding and modification. Compared with bacteria, yeast and other mammalian cells, CHO cells have the advantages of high-density culture, high expression and relatively complete protein glycosylation modification system [8].

3.2.2 Selection of transfection vector

Both plasmid transfection and viral transfection essentially introduce foreign gene EPO into host cells. However, plasmid transfection generally has low efficiency and great damage to cells. Lentiviral vectors can stably mediate gene silencing and has the advantages of high transfection efficiency and stable expression [9]. Viral vectors can also achieve selective transfection of specific cell types by changing the specificity of their outer surface proteins. This allows the researchers to selectively introduce the EPO gene into the target tissue or cell. Some viral vectors are able to form stable genomic insertions in host cells, leading to long-term gene expression.

3.2.3 Optimization of culture conditions

In host cells, the folding and modification of EPO proteins may be less than ideal, affecting their biological activity and efficacy. The use of engineered cell lines, as well as the optimization of culture conditions such as temperature, pH, nutrients, gases, etc. can promote the correct folding and modification of proteins. For example, the culture of CHO cells needs to meet certain conditions. In general, CHO cells grow best at about 37℃, while requiring 5% CO2 and a pH value of 7.2 to 7.4. In terms of nutrition, CHO cells need a medium containing sufficient nutrients such as amino acids, nucleotides, and fatty acids.

3.2.4 Product purity

Impurities may occur during the production process, affecting the purity of EPO. More efficient purification techniques such as affinity chromatography, ion exchange chromatography (IEC), etc. are used to ensure high purity of the final product. IEC is a chromatography method commonly used in the field of biochemistry. It has the advantages of high sensitivity, repeatability, good selectivity and fast analysis speed, and is widely used in the separation and purification of various biochemical substances, such as amino acids, proteins, sugars and nucleotides [10]. The principle of IEC protein separation is that under certain pH conditions, proteins with different isoelectric points carry different charges. When the separated protein solution flows through the IEC column, the protein with the opposite charge of the ion exchanger is adsorbed on the ion exchanger, and then the adsorbed protein is eluted by changing the pH or ion strength [10].

In general, methods to optimize EPO production processes need to take into account technical, economic, environmental and social factors to achieve a more efficient, cost-effective and environmentally friendly production process. This often requires interdisciplinary research and collaboration, including expertise in areas such as biotechnology, biochemistry, engineering, and environmental science.

4. Conclusion

EPO is a glycoprotein mainly produced by the kidney, which can maintain the balance of oxygen supply and demand and regulate the number of red blood cells. It is mainly used in the treatment of anemia. Its mechanism of action is to sense the level of oxygen in the body, activate relevant signaling pathways, promote the transcription and translation of relevant genes, and ultimately stimulate the production of red blood cells. The process of producing EPO drugs involves several biotechnology steps such as genetic engineering, cell culture, protein expression and modification, purification, and

preparation of preparations. The optimization of EPO production process includes selecting more suitable host cells and transfection vectors, optimizing culture conditions, and adopting more effective purification technology. By analyzing these contents, we can provide some ideas for process optimization in biopharmaceutical field. Especially for the production of protein drugs, this study can provide reference suggestions for the optimization of relevant production processes and technologies. If biotechnology applications are properly and effectively integrated into the pharmaceutical process, actual production efficiency can be improved. This study mainly analyzed the pharmaceutical process and related problems of protein drugs such as EPO, while the problems involved in the production process of other drugs were not mentioned. This study lacks examples and corresponding solutions for the special situations in the pharmaceutical process. In this study, the production of EPO was theoretically taken as an example to analyze the use of biotechnology for pharmaceutical production, and the actual production should consider technical, economic, environmental and social factors. For future research, other drugs besides protein drugs can be studied, or the parts already mentioned in this study can be refined and supplemented.

References

[1] Jiang Hengbo, Zhang Xihe, CAI Runfa et al. Application and optimization of pharmaceutical technology in the production of biological agents . Contemporary Chemical Research,2023,(16):

[2] Chen Yilun, Hui Yu, Wu Kefeng, et al. Clinical effect of erythropoietin in patients with anemia after renal transplantation . Journal of Modern Urology, 2019,28(05):424-428.

[3] Zhang Kexin, Zhu Minxia. The role of erythropoietin in the protective mechanism of cerebral ischemia and related Chinese medicine intervention . China's modern applied pharmacy, 2020, 5 (11) : 1397-1401.

[4] Xie Guiling, Wang Dongwei, Zhang Lili, et al. Effect of dexmedetomidine on JAK1/STAT1 signaling pathway during cerebral ischemia and reperfusion in rats . Medical theory and practice, 2019, 32 (8) : 1109-1110 + 1116.

[5] Qin Huaping, Yang Changchun, Wang Qiang, et al. Effect of hypothermia on hippocampal Bax expression and its relationship with apoptosis in rats with global cerebral ischemia. Of the medical journal, 2017 (12) : 1286-1289.

[6] Zheng Tianliang, Chen Xiaocui. Clinical effect of recombinant human erythropoietin combined with component blood transfusion on chronic anemia . Clinical medical research and practice, 2022, 7 (33) : 30-34.

[7] Wang Qianqian, Liu Yaqi, Qu Yan et al. Recombinant e. coli whole-cell catalysis preparation pseudouridine. Journal of biological engineering,2023, 1-14

[8] Ren Ziqiang, Wang Mengcan, Zhang Hailing et al. The research progress of CHO cells express glycoprotein . Chinese journal of biotechnology, and 2022, (6) : 54-65.

[9] Huo Yunfei, Kou Buxin, Chai Mengyin et al. Construction and functional identification of TP53BP2 shRNA lentiviral vector in vitro . Journal of Practical Hepatology, 2019,26(02):164-168.

[10] Yan Cuixia, Yin Hongrui, Shi Fangliang et al. Determination of molecular component ratio of urokinase for injection by Ion-exchange chromatography combined with molecular exclusion chromatography . Journal of Pharmaceutical Analysis, 2019,43(01):4-11.