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Study on Proinflammatory Response and Regulation of JAK2/STAT3 Signaling Pathway of Ethanol Extract of *Anacyclus pyrethrum* Root (EEAP) in Rats with Cough-Variant Asthma

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Abstract: *Objective:* To explore the modulation of the proinflammatory response and the tyrosine protein kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway in the lung of rats with cough-variant asthma (CVA) treated with ethanol extract of *Anacyclus pyrethrum* root (EEAP). *Methods:* Sixty specific pathogen-free (SPF) male Sprague-Dawley (SD) rats (with a weight range of 220–260 g, eight to ten weeks) were randomly assigned into six different groups, encompassing a control group, a model group, a positive control group (prednisone acetate, the dosage was 2.5 mg/kg, ig), the high EEAP group (640 mg/kg), medium EEAP group (320 mg/kg), and low EEAP group (160 mg/kg), with a total of 10 participants in each group. The medication was provided on a daily basis for 30 days, commencing on the 29th day. The rodents in each cohort were maintained until the 60th day. Following humane euthanasia, lung tissues and peripheral blood samples were collected. Two groups were formed by dividing rat lung epithelial type II cells: the OVA group, which received 2.5 mg/L ovalbumin stimulation and sensitization, and the treatment group, which was administered 640 mg/kg EEAP. The peripheral blood T-lymphocytes of rats in the control group and the high EEAP cohort were designated as CVA group and intervention group, respectively. The concentrations of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) were measured in each cohort using the enzyme-linked immunosorbent assay (ELISA). Utilizing real-time quantitative polymerase chain reaction (RT-PCR), the levels of IL-6, JAK2, and STAT3 were analyzed within each group. *Results:* In contrast to the control group, the concentrations of IL-1 β , IL-6, and TNF- α in the model group exhibited a substantial rise ($P < 0.05$). Moreover, the mRNA expression levels of IL-6, JAK2, and STAT3 in the experimental group were observed to be substantially elevated ($P < 0.05$). In contrast to the control group, the positive controlled group, the elevated EEAP group, and the intermediate EEAP group demonstrated substantial decreases in the mRNA expression of IL-6, JAK2, and STAT3 ($P < 0.05$), while the low EEAP group exhibited a notable decrease in the mRNA expression of JAK2. The mRNA expression levels of IL-6 and STAT3 did not exhibit any notable variation in the low EEAP group ($P > 0.05$). The concentrations of IL-1 β , IL-6, and TNF- α , along with the mRNA expression levels of IL-6, JAK2, and STAT3, demonstrated an upward trend as EEAP concentration diminished ($P < 0.05$) across the higher EEAP category, the intermediate EEAP category, and the low EEAP group. In rat type II alveolar epithelial cells, the concentrations of IL-1 β , IL-6, and TNF- α , as well as the mRNA expression levels of IL-6, JAK2, and STAT3 were diminished in the intervention

cohort in comparison to the OVA cohort ($P < 0.05$). When compared to the OVA group, the treatment group demonstrated a notable decrease in IL-1 β , IL-6, and TNF- α , along with mRNA expression levels of IL-6, JAK2, and STAT3 ($P < 0.05$). In contrast to the CVA group, the intervention group demonstrated a significant reduction in IL-1 β , IL-6, and TNF- α levels, along with the mRNA expression levels of IL-6, JAK2, and STAT3 in T lymphocytes ($P < 0.05$). *Conclusion:* The study demonstrated that EEAP effectively suppressed the proinflammatory response mediated by IL-6 and TNF- α , along with the JAK2/STAT3 pathway, in the lungs of rats suffering from cerebral CVA.

Keywords: Ethanol extract of *Anacyclus pyrethrum* root (EEAP); Proinflammatory response; Tyrosine protein kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway; Cough-variant asthma (CVA)

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1. Introduction

Cough-variant asthma (CVA) represents a unique form of bronchial asthma, distinguished by persistent coughing in the absence of wheezing manifestations [1]. Over the past few decades, there has been a notable rise in the occurrence of CVA cases globally, and the prevalence of pediatric CVA has progressively risen. Without prompt intervention, certain CVA cases may progress to characteristic asthma symptoms [2]. Currently, there are numerous medications available for the management of CVA [3]. Nevertheless, the adverse effects of these medications have led to a decrease in patient adherence. Therefore, it becomes crucial to identify options with superior therapeutic attributes [4].

Anacyclus pyrethrum, a time-honored Chinese herbal remedy, primarily comprises flavonoids, polysaccharides, volatile oils, tannins, amino acids, and other constituents. It has a positive therapeutic impact [5]. Recent research has demonstrated that EEAP exhibits anti-inflammatory properties and has been proven to mitigate oxidative stress-induced injury [6]. Nevertheless, the molecular mechanism of EEAP efficacy in managing CVA is still a mystery. Within this research paper, we investigated the possible role of EEAP in dampening pulmonary inflammatory reactions in CVA, both *in vivo* and *in vitro*. This paper aimed to establish a theoretical foundation for the practical implementation of EEAP in the management of CVA.

2. Materials and methods

2.1. Materials

2.1.1. Instruments

Gongyi Yuhua Instrument Co., LTD., China, produces the R-2003 rotary evaporator. Gongyi Yuhua Instrument Co., LTD., located in Gongyi, China, offers the R-2003 water bath. China's Zhengzhou Great Wall Technology Industry and Trade Co., LTD. manufactures the SHB-III recirculating water-type multi-purpose vacuum pump, a versatile device that finds applications in various industries. Shanghai Yiheng Instrument Co., LTD., China, offers the DZF-6090 vacuum drying oven. Full wavelength microplate reader (Biotek, Epoch, USA); Leica Corporation, located in China, produces the DMI4000B fluorescence inverted microscope. RT-PCR device, KS18 biosafety cabinet (Thermo Fisher Scientific); Galaxy 170R CO₂ incubator (New Brunswick Scientific); Electrophoresis equipment (BIO-RAD); Electronic scale (Sartorius, Germany); high-speed refrigerated centrifuge available (3-18 K, Sigma).

2.1.2. Preparation of drug products

The root of *Anacyclus pyrethrum* was sourced from Xinjiang Bencao Tang Co., LTD (batch number: 20210901) and was recognized as the origin of *Anacyclus pyrethrum* (L.) DC. within the family Asteraceae by Shalameti Mati (director and pharmacist at Xinjiang Institute for Food and Drug Control). In the course of the investigation, a suitable quantity of *Anacyclus pyrethrum* (L.) was collected, ground, and the proportion of solid to liquid was determined to be 1:6 (the solution had a concentration of 6 g/mL), and it was obtained through reflux extraction using 65% ethanol at a temperature of 50°C, with the process being repeated three times for a duration of two hours. The sample underwent a series of processing steps, including filtration, integration, concentration via a rotary evaporator, freeze-drying to obtain powder form, and subsequent storage in a refrigerator maintained at 4°C until its intended application. Acetate prednisone tablets were obtained from Tianjin Tianyao Pharmaceutical Company, Limited (5 mg, Lot: 2109027), while sodium chloride injection was sourced from Sichuan Kelun Pharmaceutical Co., LTD (0.9 g:100 ml, Lot: C22011802).

2.1.3. Reagents

Type II alveolar epithelial cell RLE-6TN can be obtained from Shanghai Binsui Biotechnology Co., LTD. The provided sentence fragment can be rewritten as follows: A quantity of 500,000 cells is present in each T25 culture bottle, with a purity level of no less than 99%, and the batch number is 2022011325. Takara was the source of the Trizol reagent, which came in a 100 ml bottle with the lot number 9909020. Sigma (48T, Lot: 1002638271) supplied ovalbumin (OVA) for this study. Capsaicin was acquired from Sigma Corporation, with a batch number of 102711388. A 10% solution of Freund's adjuvant was obtained from Sigma Corporation (Lot: 10025515070). ELISA kits for interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) were acquired from Shanghai Biyuntian Biotechnology Co., LTD (96T, Lot: 041814002105000418). The construction of primers for IL-6, JAK2, and STAT3 was carried out by Beijing Sangon Company. The DMEM medium (with 10% FBS) was obtained from Wuhan Punosay Life Technology Co., LTD (125 ml, Lot: WHO11122121SP08), chloroform, 100% ethanol (all analytically pure), and purified water.

2.1.4. Animals

The animal source for this study consists of specific pathogen-free (SPF) male Sprague-Dawley (SD) rats, which were obtained from the Shanghai Slaker Laboratory Animal Center (license number: SCXK: 2022-0004). These rats were aged between 6 and 8 weeks and had a weight range of 220 to 260 g. The rats were kept in the Shanghai Slake Laboratory Animal Center. The surrounding temperature ranged between 20°C and 24°C, with relative humidity varying between 50% and 60%. The day and night cycle was maintained at a duration of 12 hours, and water was provided freely. All protocols associated with this study were in compliance with the ethical guidelines sanctioned by the hospital Ethics Committee (Approval No. 2022-05-01).

2.2. Methods

2.2.1. Experimental animals and grouping scheme

The rodents were provided unrestricted access to hydration and sustenance ^[7]. In the OVA model group, rats were sensitized using a 1 mg/mL freshly prepared OVA solution, which consisted of 1% normal saline solution mixed with

10% complete Freund's adjuvant solution. Ten positions were selected on the hind foot, groin, waist, back, and neck of each rat. Each point received an injection of 0.05 mL, while 0.5 mL was intraperitoneally administered simultaneously, resulting in a total volume of 1 mL [8]. Following a week's interval, the rodents were subjected to another feeding session to reinforce the sensitization process, which was then maintained for a duration of 14 days. In the study involving animals, the rodents were allocated into six distinct groups: a control group (administered normal saline), a model group, a positive control group (treated with prednisone, ig, at a dosage of 2.5 mg/kg), the high (640 mg/kg), medium (320 mg/kg), and low (160 mg/kg) EEAP groups were administered through gavage, with the dosage determined based on the alcohol extract's quality. Water was utilized as the dissolving medium, the dosage was established based on the preceding acute toxicity study conducted by EEAP and the findings of relevant research [9]. On the 15th day following the creation of the model, the rodents were housed within a custom-built transparent aerosol chamber. Daily administration of a 1% OVA solution under constant pressure of 400 mmHg (1 mmHg equivalent to 0.133 kPa) for 20 minutes, over a period of 15 days, was employed to induce asthma symptoms. For 30 consecutive days, beginning on day 30, the rats were administered OVA on a daily basis. Over a period of 60 days, the rodents in each cohort were provided with sustenance and subsequently humanely dispatched. Concurrently, lung tissue samples and peripheral blood specimens were obtained. Lung tissue was utilized to assess the presence of inflammatory factors and JAK2/STAT3 signaling molecules, while peripheral blood samples were employed to isolate T lymphocytes.

2.2.2. Culture and grouping of rat lung epithelial type II cells

The epithelial type II cells of rat lung, designated as RLE-6TN, were propagated in a DMEM growth medium supplemented with 10% fetal calf serum (FBS) and antimicrobial agents (100 U/ml penicillin and streptomycin), under an incubator condition maintained at 37°C, 5% CO₂, humid and sterile. Cells in the logarithmic growth stage were chosen and partitioned into two categories: OVA group (subject to 2.5 mg/L OVA stimulation and sensitization) and treatment group (exposed to 640 mg/kg EEAP). Following a duration of 24 hours for the treatment process, both the cells and the culture supernatant were collected. The investigation focused on the detection of JAK2/STAT3 signaling pathway components and the quantification of inflammatory mediators. The previous findings of ELISA analysis of inflammatory mediators in the plasma of CVA rats indicated that EEAP can decrease the levels of IL-6 and TNF- α , and the impact was displayed in a dose-responsive manner. Thus, the dosage plan was established upon the findings of the preceding *in vivo* study, and the higher dosage was chosen as the therapeutic group [10].

2.2.3. Isolation of rat peripheral blood T lymphocytes

In the model group and high EEAP group (640 mg/kg) of section 2.2.1. (prior to the termination of 60-day-old rats), T-lymphocytes were isolated from freshly collected anticoagulated peripheral blood of rats. Following gradient centrifugation, isolated nucleated cells were suctioned, and T-lymphocytes were procured utilizing nylon hair columns, and T-lymphocytes derived from the model group and the high EEAP group (640 mg/kg) were designated as the CVA and the therapeutic groups respectively. The subgroups were administered the respective doses as outlined above. RPMI 1640 culture medium supplemented with 10% FBS was used to cultivate T cells for a duration of 24 hours at a temperature of 37°C and an atmosphere of 5% CO₂. Cell culture supernatant was obtained for the purpose of identifying

the presence of JAK2/STAT3 signaling pathway components, as well as assessing the concentration of inflammatory mediators.

2.2.4. Enzyme-linked immunosorbent assay

Supernatants were obtained from rat lung tissue homogenates that underwent centrifugation in each group, including the T lymphocyte CVA group and the treatment group, and from rat lung epithelial type II cell OVA group and control group. The concentrations of IL-1 β , IL-6, and TNF- α were measured following the experimental protocol outlined in the ELISA kit manual. The absorption level was determined at a wavelength of 450 nm utilizing an enzyme indicator (Biotek Epoch, United States).

2.2.5. Real-time quantitative PCR Analysis (qRT-PCR)

Total RNA extraction was performed by incorporating Trizol reagent (as per the manufacturer's guidelines) into the supernatant obtained from centrifugation of rat lung tissue homogenates from each respective group. The supernatant of the T-lymphocyte CVA group and the therapy cohort, and the supernatant obtained from the rat lung epithelial type II cells in the OVA group and the control group. The RNA was converted into cDNA for quantitative PCR analysis. The parameters for PCR amplification were as follows: Before denaturation, the temperature is set to 95°C for a duration of 1 minute. This is then followed by 30 successive cycles, each consisting of denaturation at 95°C for a duration of 30 seconds, annealing at 58°C for a duration of 5 seconds, and a final extension at 72°C for a duration of 5 seconds. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the standard gene for quantifying mRNA levels of IL-6, JAK2, and STAT3, and every gene in every sample was replicated thrice. The accuracy of PCR outcomes was verified through the dissolution curve method. The difference in Ct values for the target gene and the internal reference is calculated as $\Delta Ct = Ct \text{ (target gene)} - Ct \text{ (internal reference)}$. The difference in ΔCt between the experimental group and the control group is then determined as $\Delta\Delta Ct = \Delta Ct \text{ (experimental group)} - \Delta Ct \text{ (control group)}$. The analysis of target gene expression was conducted using the $2^{-\Delta\Delta Ct}$ method. The qRT-PCR primer sequence (5'-3') was composed of the following components: IL-6 forward primer: TGAGGCGAAGTCTGAATTG, and reverse primer: CTACCCGTTGCCCTATTACT; GAPDH forward: ACCGGATATGCTCTTATTCGG, reverse: CCACTCCTAGATGATTGTGAG; JAK2 forward: TGCTACGTATACGTGTCC, reverse: CACTGGCATCGAGGCGT; STAT3 forward: CTCACCTTTGGCACCACA, reverse: TACGCTTCTCGACTTTGATT.

2.3. Statistical analysis

Data analysis was performed using GraphPad Prism 7.0 statistical software, and a statistical diagram was generated. The comparative expression levels of normal distribution measurement data, including IL-1 β , IL-6, TNF- α , and JAK2. The expression of p-JAK2 and STAT3 was presented as mean \pm standard deviation (SD). A *t*-test was employed to compare T lymphocytes between the CVA group and treatment group, as well as the OVA group and treatment group in the RLE-6TN group. To analyze the given data, a one-way analysis of variance (ANOVA) was employed for conducting multiple comparisons. Furthermore, the LSD-*t* test was utilized for evaluating pairwise comparisons. The examination threshold was set at $\alpha = 0.05$, and a *P*-value less than 0.05 was deemed statistically significant.

3. Results

3.1. Effects of EEAP on inflammatory factors in lung tissue of CVA model rats

In contrast to the control group, the concentrations of IL-1 β , IL-6, and TNF- α in the model group exhibited a substantial rise ($P < 0.05$). In contrast to the CVA model group, the prednisone acetate cohort exhibited a higher response in the context of medium and low-dose EEAP administration, notable decreases in IL-1 β , IL-6, and TNF- α concentrations were observed ($P < 0.05$). In contrast to the prednisone acetate cohort, the high-dose EEAP group exhibited no notable variations in IL-1 β , IL-6, and TNF- α ($P > 0.05$). Both the medium-dose EEAP group and the low-dose EEAP group exhibited substantial rises in IL-1 β , IL-6, and TNF- α levels ($P < 0.05$). Furthermore, the concentrations of IL-1 β , IL-6, and TNF- α in the high-dose EEAP cohort, the medium-dose EEAP group and the low-dose EEAP group showed an increase in their respective concentrations ($P < 0.05$) as the EEAP concentration decreased. See **Figure 1**.

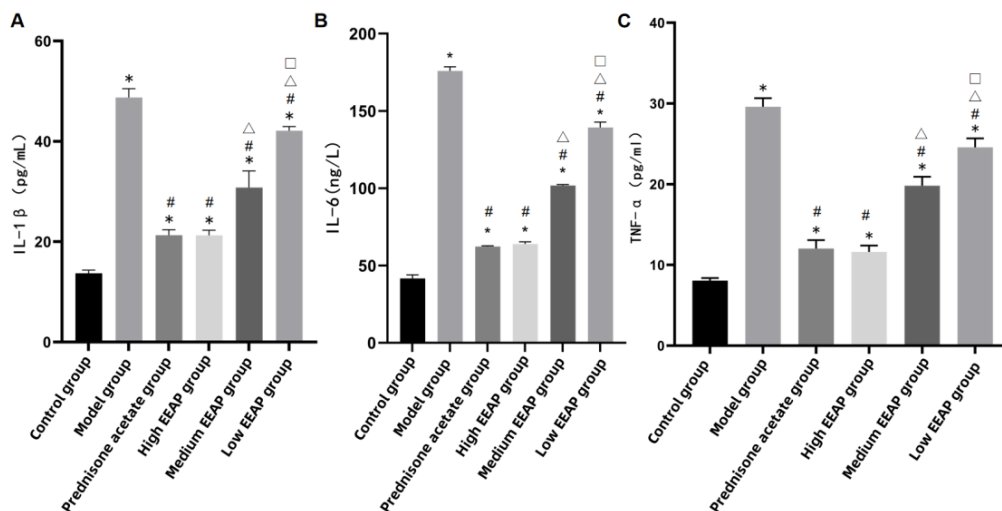


Figure 1. Analysis of the mRNA expression levels of IL-1 β , IL-6, and TNF- α in lung tissues across different groups. A series of experiments were conducted using the ELISA technique to assess the expression levels of three key inflammatory markers, namely IL-1 β , IL-6, and TNF- α , in lung tissues obtained from different experimental groups. In comparison to the control group, * $P < 0.05$; In contrast to the control group, # $P < 0.05$; Compared with the high EEAP group, $\Delta P < 0.05$. Compared with the middle EEAP group, $\square P < 0.05$.

3.2. Effect of EEAP on activation of JAK2/STAT3 signaling pathway in lung tissue of CVA model rats

In contrast to the control group, the model group exhibited significantly elevated concentrations of IL-1 β , IL-6, and TNF- α ($P < 0.05$). In contrast to the CVA model group, the concentrations of IL-1 β , IL-6, and TNF- α levels were notably reduced in the prednisone acetate cohort, as well as in the high-, medium-, and low-dose EEAP groups ($P < 0.05$). In the high-dose EEAP group, there were no statistically significant differences in the levels of 1 β , IL-6, and TNF- α ($P > 0.05$). However, the levels of IL-1 β , IL-6, and TNF- α in the medium-dose EEAP group and the low-dose EEAP group were notably elevated ($P < 0.05$). As the concentration of EEAP decreased, the low-dose EEAP group experienced an increase ($P < 0.05$). See **Figure 2**.

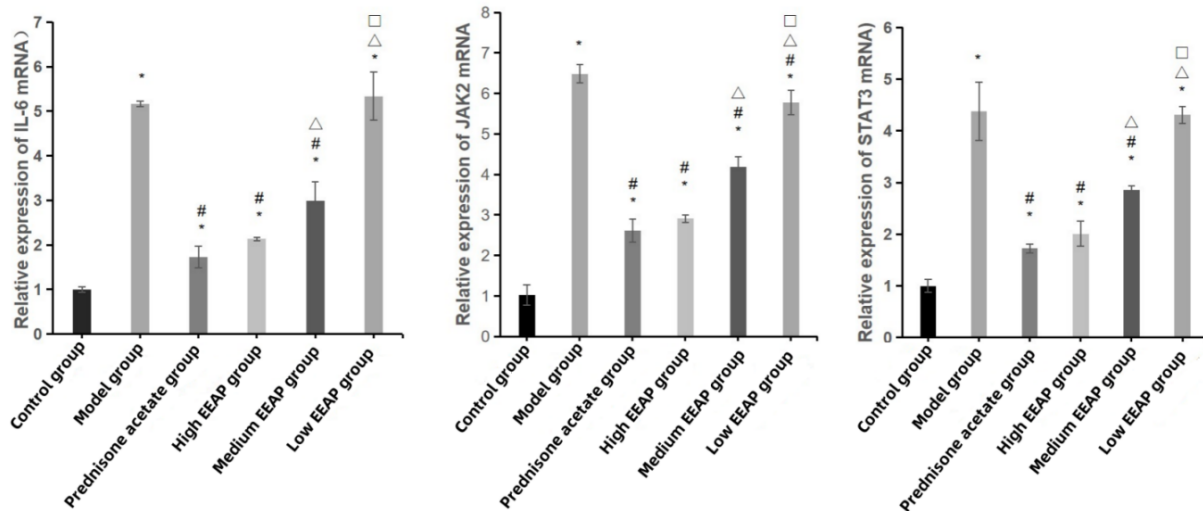


Figure 2. Comparison of mRNA expression levels of IL-6, JAK2, and STAT3 in lung tissues from various experimental groups. (A) ~ (C) Quantitative real-time polymerase chain reaction (qRT-PCR) technique was employed to measure the mRNA expression levels of IL-6, JAK2, and STAT3 in lung tissues derived from various experimental groups. In comparison to the control group, * $P < 0.05$; In contrast to the control group, # $P < 0.05$; Compared with the high EEAP group, $\Delta P < 0.05$. Compared with the middle EEAP group, $\square P < 0.05$.

3.3. Effect of EEAP on inflammatory factors in OVA-stimulated lung epithelial cells and T lymphocytes of CVA rats

In contrast to the OVA group, the treatment group exhibited substantially reduced concentrations of IL-1 β , IL-6, and TNF- α in lung epithelial cells ($P < 0.05$). In contrast to the CVA group, the concentrations of IL-1 β , IL-6, and TNF- α in T lymphocytes within the treatment group exhibited a substantial reduction ($P < 0.05$). See **Figure 3**.

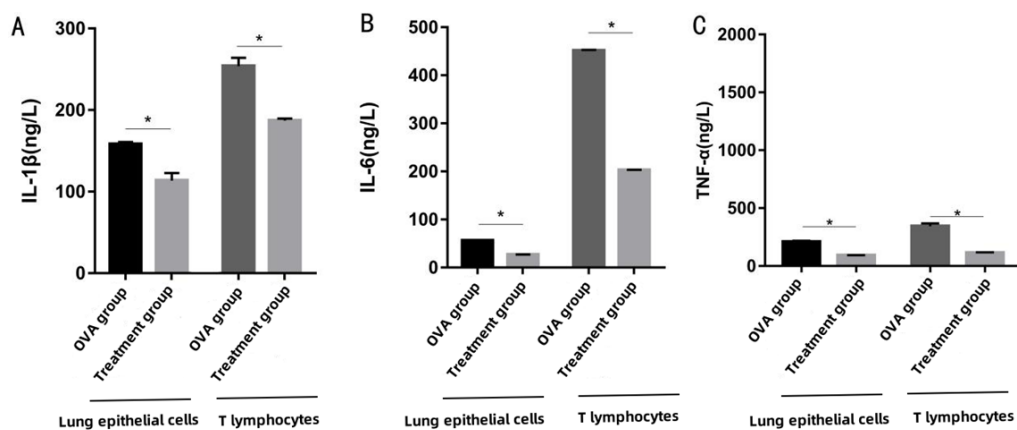


Figure 3. Comparison of the expression levels of IL-1 β , IL-6, and TNF- α in the culture medium of cellular samples from various groups. (A) ~ (C) Enzyme-linked immunosorbent assay (ELISA) was employed to measure the expression levels of IL-1 β , IL-6, and TNF- α in the various cell groups. * $P < 0.05$.

3.4. Effect of EEAP on JAK2/STAT3 signaling pathway in lung epithelial cells and T lymphocytes of rats with cough-variant asthma

In contrast to the OVA group, mRNA levels of IL-6 in the treatment group were significantly reduced. In the lung epithelial cells of the OVA treatment group, JAK2 and STAT3 levels were notably reduced ($P < 0.05$). In contrast to the CVA group, the mRNA expression of IL-6 in the treatment group was significantly reduced. In the T lymphocytes of the treatment group, both JAK2 and STAT3 exhibited substantial reduction ($P < 0.05$). See **Figure 4**.

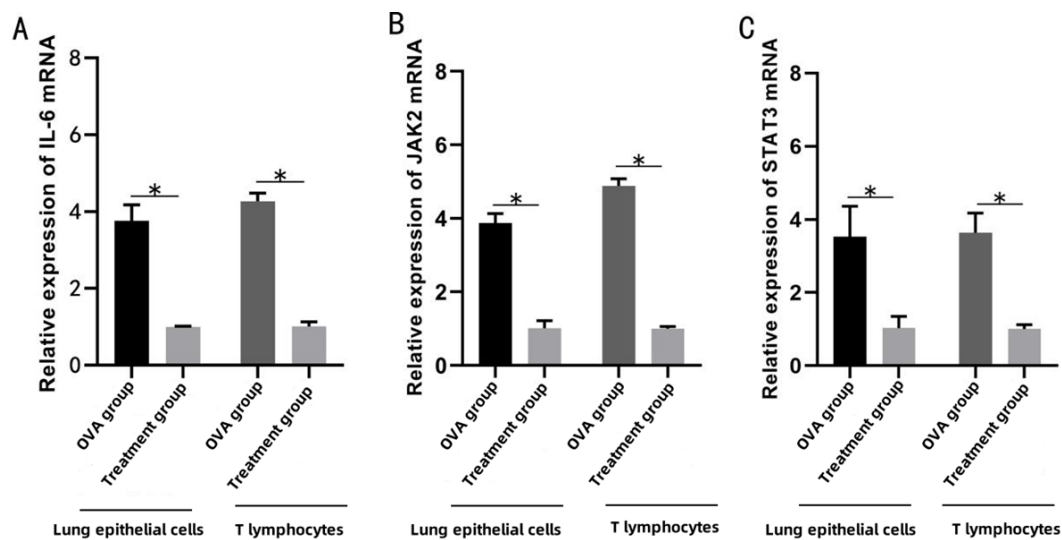


Figure 4. Analysis of mRNA expression levels for IL-6, JAK2, and STAT3 in various cellular groups. Note: (A) ~ (C) Quantitative real-time polymerase chain reaction (qRT-PCR) technique was employed to measure the mRNA expression levels of IL-6, JAK2, and STAT3 in various cell groups. * $P < 0.05$.

4. Discussion and conclusion

CVA is noted to occur independently and is usually defined by a chronic, enduring cough, throat irritation, and the absence of wheezing [11]. Moreover, it has been noted that CVA occurs in individuals with asthma as well. Despite its non-life-threatening nature, CVA exerts a substantial influence on the patient's daily living experience [12]. It is crucial to acknowledge that no particular drugs are currently prescribed for CVA. Therefore, further investigation into the management of CVA is essential. Studies have shown that persistent airway inflammation is a characteristic feature of CVA. The severity of this condition can be measured by evaluating peripheral blood eosinophil counts and conducting anti-hyaluronidase reaction tests. Recent investigations have shown that upon translocation to inflamed tissue and activation, T lymphocytes emit a range of cytokines and inflammatory factors, such as IL-6 and IL-1 β . IL-12 is a substance that has the ability to cause inflammation in the airways [13]. Within this research paper, it was verified that EEAP suppressed inflammation in rat lungs and decreased the expression levels of IL-1 β and IL-6. TNF- α is found in lung tissue, lung epithelial cells, and T lymphocytes.

In this study, a rat model was employed to explore the mechanism of action of EEAP in cough-variant asthma. *In vivo* studies demonstrated that EEAP showed a tendency towards inflammatory response and immunomodulation in the CVA rat model. IL-6 and TNF- α have been demonstrated to be crucial in the pathogenesis of asthma [14]. IL-6 has been discovered to play a role in controlling inflammatory and immune reactions, Numerous studies have provided evidence indicating that a certain substance impacts cellular functionality by activating the JAK2/STAT3 signaling pathway [15]. Moreover, IL-1 β and TNF- α have been recognized as crucial pro-inflammatory mediators capable of eliciting airway inflammation and bronchospasm [16]. Moreover, the investigation demonstrated that EEAP reduced the expression levels of IL-1 β , IL-6, and TNF- α in OVA-stimulated lung epithelial cells and T-lymphocytes in the CVA group. This discovery highlights the crucial role of EEAP in the treatment of lung inflammation associated with CVA.

Moreover, our research revealed that EEAP can exert its anti-inflammatory impact on stroke model rats by reducing TNF- α production and limiting inflammatory cell invasion [17]. STAT3 transcription factors, belonging to the STAT family, play a crucial role in numerous biological processes. Numerous studies indicate that STAT3 is crucial in the development of asthma [18]. STAT3 is activated in peripheral blood mononuclear cells and airway smooth muscle tissues of individuals with asthma, and this activation is linked to an increase in cytokines [19]. Blocking STAT3 signaling reduces airway inflammation [20], thereby reversing the adverse impacts of CVA. The JAK/STAT signaling pathway plays a crucial role in the development of asthma. The intracellular signaling pathway known as JAK/STAT facilitates the transmission of chemical messages from the cell membrane to gene promoters localized within the nucleus [21]. JAK/STAT frequently interacts with signaling pathways such as PI3K/AKT, NF- κ B, HIF1 α , and others, leading to the generation of diverse intricate biological responses [22]. In this study, we demonstrated that EEAP disrupted JAK2 expression and suppressed upstream IL-6 signaling following cell incubation *in vitro*. The evaluation also demonstrated that the control group exhibited markedly increased mRNA expression of IL-6, JAK2, and STAT3 were observed in the EEAP treatment group *in vivo*, where there was a substantial decrease in levels of IL-6, JAK2, and STAT3 mRNA at medium and high doses. This investigation indicated that EEAP may exhibit its anti-inflammatory properties by suppressing the JAK2/STAT3 signaling pathway. In the future, our research team will aim to delve into the impact of lung inflammation on cognitive function and focus on the JAK2/STAT3 signaling pathway specifically.

There are certain limitations in this research. Initially, the investigation was conducted solely using a rat model. Further animal experimentation and clinical trials are required to corroborate the findings of this research. In investigating the induction of JAK2/STAT3 signaling pathway by EEAP in lung tissues of CVA model rats, the expression levels of IL-6 and STAT3 mRNA in the low EEAP group did not exhibit a statistically significant difference when compared to the model group. The cause of this requires further exploration. Secondly, we have not conducted an in-depth analysis of the standalone impacts of EEAP and only examined the impact on inflammatory mediators and signaling pathways in the CVA model. Hence, further investigation and enhancement of the dosage regimen are necessary.

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Disclosure statement

The authors declare no conflict of interest.

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Research Progress and Application of Lipid-Lowering Drugs and Pharmacogenomics in Managing Chronic Diseases in the Elderly

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Abstract: With the advent of an aging society in China, the elderly population is growing, and their health status and disease burden are also increasing. Cardiovascular and cerebrovascular diseases are the main killers that harm the health of the elderly. Dyslipidemia is one of the independent risk factors for the occurrence of cardiovascular and cerebrovascular diseases. Lipid-lowering drugs, as commonly used drugs to prevent cardiovascular events in clinical practice, are widely used in elderly patients. This article summarizes and categorizes several major types of drugs currently used for lipid-lowering therapy, combining the latest research progress in pharmacogenomics to provide references for lipid-lowering regimens in elderly patients.

Keywords: Elderly; Chronic disease management; Lipid-lowering drugs; Pharmacogenomics

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1. Introduction

With the improvement of living standards and the acceleration of population aging in China, patients' requirements for health care are also increasing. Especially among elderly patients with chronic diseases, due to the diverse and complex types of diseases and the association of many diseases with genes, more detailed classification and targeted intervention are required [1]. However, there are few studies on chronic diseases of the elderly in China. How to meet the needs of personalized diagnosis and treatment of chronic diseases, optimize drug treatment plans, and ensure long-term safety and effectiveness of patients are still key issues that need attention.

In recent years, with the development of pharmacogenomics, personalized diagnosis and treatment of lipid-lowering drugs have become possible. Through correlation analysis of gene polymorphism and drug efficacy, precise drug administration can be facilitated, thereby achieving better treatment effects and reducing toxic side

effects. In recent years, there have been many breakthroughs in research on lipid-lowering drugs based on pharmacogenomics. For example, studies have shown that gene polymorphisms of *SLCO1B1* are associated with statin-induced myopathy, and gene polymorphisms of *APOE* can also affect the lipid-lowering effect of statins [2,3]. Further research has found that variations in the *PCSK9* gene are related to the efficacy of PCSK9 inhibitors. These findings suggest that genetic testing is expected to provide individualized lipid-lowering treatment regimens for elderly patients, thereby optimizing treatment effects and reducing the occurrence of adverse reactions. However, despite significant progress in the field of lipid-lowering drugs in pharmacogenomics, their application in the management of chronic diseases in the elderly still faces many challenges. Elderly patients often have multiple chronic diseases and need to use multiple drugs simultaneously, which complicates drug interactions. This increases the difficulty of implementing individualized drug regimens based on genetic testing. In addition, the high cost of pharmacogenomic testing technology limits its widespread application in primary medical institutions and economically underdeveloped areas. At the same time, the varying levels of clinicians' and pharmacists' knowledge of pharmacogenomics also affect its promotion and application in clinical practice.

2. Overview of pharmacogenomics

Pharmacogenomics primarily studies the effects of drugs on individuals with different genotypes. Currently, multiple reference genomes are used to guide clinical drug administration, including the International HapMap Project, gene frequency distribution characteristics of the Chinese population, and common single-nucleotide polymorphisms (SNPs) in European and American populations. Among them, the genes closely related to cardiovascular diseases are *APOE*, apolipoprotein E, *PCSK9*, and acyl-CoA binding protein genes.

The *APOE* gene plays a crucial role in regulating high-density lipoprotein (HDL) levels, liver cholesterol metabolism, and the formation of atherosclerosis. The *APOE* gene can mediate the clearance of low-density lipoprotein (LDL) by the liver through its encoded apolipoprotein A2, allowing HDL to be enriched [4]. The *PCSK9* gene inhibits the degradation of LDL receptors in the liver, leading to a continuous increase in LDL-C and ultimately causing atherosclerotic lesions in arteries. The acyl-CoA binding protein gene is mainly involved in the body's lipid metabolism through its encoded acetyl-CoA carboxylase. Mutations or variations in these genes can increase the risk of cardiovascular and cerebrovascular diseases.

3. Types of lipid-lowering drugs and genomics

Cholesterol is an important lipid component of the cell membrane and exerts its physiological functions mainly through the action of intracellular β -lipoproteins and apolipoproteins (ApoB). There are a large number of cholesterol receptors on the cell surface that transport cholesterol to the endoplasmic reticulum membrane for storage or secretion into bile, liver, and adipose tissue. It is then released into the bloodstream in the form of very low-density lipoproteins. When the total serum cholesterol (TC) exceeds 10.3 mmol/L, it is considered hypercholesterolemia. Currently, commonly used lipid-lowering drugs in clinical practice include statins, nicotinic acid, fibrates, and resins.

3.1. Statins

As an important class of lipid-lowering drugs, statins primarily work by inhibiting the activity of

3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, thereby reducing endogenous cholesterol synthesis and effectively lowering low-density lipoprotein cholesterol (LDL-C) levels. At the same time, they can also slightly increase high-density lipoprotein cholesterol (HDL-C) and lower triglycerides (TG) [5]. Numerous clinical studies have confirmed that statins can effectively reduce the incidence of ASCVD and are considered cornerstone drugs for lipid-lowering therapy. In genomics, genetic variations in the *SLCO1B1* gene have a significant relationship with the plasma concentration and adverse effects of statins.

The gene encodes the organic anion transporting polypeptide 1B1 (OATP1B1), which is involved in the transport of statins from the blood to the liver [6]. Individuals carrying the *SLCO1B1**5 allele have reduced OATP1B1 transport function, leading to increased blood concentrations of statins and a significantly increased risk of muscle toxicity, such as a higher probability of developing rhabdomyolysis [7]. Additionally, polymorphisms in genes such as *ABCC2* and *CYP3A4* can also affect the pharmacokinetics and pharmacodynamics of statins, resulting in differences in efficacy and safety among individuals.

3.2. Fibrate drugs

Fibrate lipid-lowering drugs are divided into two major categories: niacin and fenofibrate. Fenofibrate can reduce cholesterol formation by inhibiting the activity of HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis, and is a commonly used drug for the treatment of mild to moderate hypercholesterolemia. Niacin, on the other hand, is a vitamin with lipid-lowering effects that can increase HDL and regulate the lipid profile, making it a safer drug for the treatment of myopathy and renal failure.

Fibrate drugs primarily work by activating peroxisome proliferator-activated receptor alpha (PPAR α), regulating the expression of genes involved in lipid metabolism, promoting the catabolism of triglycerides (TG), significantly reducing TG levels, and moderately increasing HDL-C and decreasing LDL-C. They are suitable for patients with primarily high TG hyperlipidemia or mixed dyslipidemia, and they play a role in reducing the risk of ASCVD while lowering TG-rich lipoprotein levels.

Genomic studies have found that polymorphisms in the *PPARA* gene can affect the efficacy of fibrate drugs. For example, the rs4253778 polymorphism in the *PPARA* gene is associated with the effect of fibrate drugs on reducing TG, and there are differences in drug response among patients with different genotypes [8]. Additionally, polymorphisms in the *APOC3* gene, which encodes a protein that inhibits lipoprotein lipase activity, can also influence the lipid-lowering effect of fibrate drugs, potentially altering their regulatory effect on TG metabolism.

3.3. Resin drugs

Resin drugs, also known as bile acid sequestrants, mainly include bile acid derivatives, polyunsaturated fatty acids, and monoamine oxidase inhibitors. Bile acid derivatives primarily work by competitively binding to cholesterol transporters on the surface of liver cells, thereby promoting the excretion of cholesterol from the body. Polyunsaturated fatty acids mainly reduce blood cholesterol concentrations by promoting the reverse transport of endogenous cholesterol and increasing the excretion of free cholesterol. However, long-term use may increase the risk of arterial hardening and cardiovascular events. Monoamine oxidase inhibitors lower blood cholesterol levels by inhibiting the action of methylmalonyl-CoA oxidase in the polyamine metabolic pathway.

In the field of genomics, there are relatively few studies on resin drugs. However, some research suggests that

polymorphisms in the *ABCG5/ABCG8* genes may affect the regulatory role of resin drugs on cholesterol metabolism. The ABCG5 and ABCG8 proteins are involved in intestinal cholesterol absorption and biliary cholesterol secretion, and variations in these genes may alter the intestine's ability to process cholesterol, thereby influencing the lipid-lowering effects of resin drugs [9].

3.4. Ezetimibe

Ezetimibe selectively inhibits the absorption of intestinal cholesterol by targeting the Niemann-Pick C1-Like 1 (NPC1L1) protein at the brush border of the small intestine, thereby lowering blood cholesterol levels. Ezetimibe can be used in combination with statins to synergistically reduce LDL-C levels. This combination therapy has been shown to be more effective in lipid lowering than monotherapy and has a good safety profile [10].

Genomic studies have revealed that polymorphisms in the *NPC1L1* gene significantly affect the efficacy of ezetimibe. Individuals carrying specific *NPC1L1* gene variations may experience a reduced inhibitory effect on intestinal cholesterol absorption, leading to a decreased lipid-lowering response to ezetimibe. Additionally, polymorphisms in the *ABCG5/ABCG8* genes may indirectly influence the efficacy of ezetimibe by affecting intestinal cholesterol transport and metabolism [11].

4. Drug targets in genomics and prediction methods

Identifying the key genes that affect drug efficacy and toxicity is a critical step in pharmacogenomics, as part of the study of different genetic factors. Reports have shown that gene variations, including ApoE, Lp(a), and PCSK9, can reduce patient responsiveness to statins, while having no significant impact on other lipid-lowering drugs such as ezetimibe and fibrates.

Therefore, it is speculated that this is related to whether the above gene variations lead to changes in the expression level of drug-metabolizing enzymes. For example, increased CYP3A4 activity, elevated CYP substrate specificity, and an increase in CYP degradation products have been observed in patients with *APOE* gene mutations. Meanwhile, the CYP2C19rs7265048 locus is also associated with statin metabolism. Polymorphism at this locus is positively correlated with the therapeutic effect of statins, meaning that individuals carrying this polymorphism show a more significant increase in plasma drug concentration and better drug efficacy after administration. Additionally, mutations in the *LpA* gene or decreased LpA protein levels can affect the beta-oxidation process of fatty acids in the liver, leading to increased cholesterol synthesis and ultimately causing dyslipidemia [12].

Currently, it is known that multiple drugs have potential interactions. For instance, clopidogrel interacts with liver enzyme inducers (LTP), limiting its clinical application. Dipyridamole can interact with rifaximin, simvastatin, fluvastatin, etc., potentially causing an increase in transaminase levels. When fenofibrate is used in combination with hypoglycemic drugs or anticoagulants, it may lead to poor glycemic control due to antagonistic effects.

Some scholars have proposed methods to predict drug mechanisms using bioinformatics tools, employing techniques such as bibliometric analysis, machine learning, and correlation analysis for data processing and mining. A series of mathematical models have been established to evaluate the relationship between specific gene variations and drug responses, providing an important basis for guiding individualized medication. Recently, researchers have selected a large number of differentially expressed genes (DEGs) from vast genetic datasets and constructed a new deep learning-based prediction model based on these DEGs. This model can effectively distinguish changes in

individual gene expression levels and their underlying causal effects, thereby predicting the potential mechanisms of multiple drugs or therapies. This will significantly accelerate the speed of targeted drug development and improve drug development efficiency.

5. Summary and outlook

The acceleration of population aging has made dyslipidemia a common chronic disease among the elderly. Compared to the general population, elderly individuals with high cholesterol tend to have faster disease progression and higher mortality rates. Commonly used lipid-lowering drugs include statins, fibrates, and niacin. In recent years, pharmacogenomics-based research methods have enabled the analysis of the mechanism of action of potential drugs and assisted clinicians in selecting the most suitable medications for patients, thus achieving personalized medication. However, current pharmacogenomics research suffers from a limited sample size and a lack of large-scale clinical trials for validation. Larger, multicenter clinical studies are needed to confirm these findings. Additionally, individual differences in drug response, metabolic enzyme polymorphisms, and genetic instability can significantly impact drug efficacy, making it challenging to conduct precise individualized drug screening based on a single target or pathway. Therefore, it is essential to comprehensively analyze existing research results based on patients' individual characteristics and establish a more comprehensive and efficient pharmacogenomic database through correlation studies across multiple databases. On this foundation, by combining genomics technology with artificial intelligence techniques, a drug-gene interaction network based on machine learning can be constructed. This network can then be used to automatically recommend medication regimens through predictive models, representing a significant direction for future pharmacogenomics research. With the continuous development of pharmacogenomics-related technologies, it is believed that they will provide strong support for rational drug use in clinical settings, allowing elderly patients to benefit from safer and more effective drug treatments ^[13].

Disclosure statement

The author declares no conflict of interest.

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The Critical Role of Impurity Analysis in Quality Control During Drug Testing and Its Optimization Paths

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Abstract: Impurity analysis in drug testing plays a crucial role. It can identify and control harmful impurities, ensuring drug purity, stability, and safety while guaranteeing efficacy. Currently, various techniques and methods are employed for impurity analysis, including chromatographic techniques such as ion chromatography, gas chromatography, and high-performance liquid chromatography, as well as mass spectrometry techniques like liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry, and spectrophotometric methods like UV-visible spectrophotometry. However, impurity analysis techniques face challenges, including the need to improve sensitivity and accuracy, overcome difficulties in analyzing complex sample impurities, and address method reproducibility issues. Optimizing impurity analysis involves introducing advanced techniques like ultra-high-performance liquid chromatography and chromatography-mass spectrometry, establishing standardized processes, strengthening personnel training, and utilizing artificial intelligence and automation technology. These efforts enhance drug quality control levels, ensuring safe and effective medications.

Keywords: Drug testing; Impurity analysis; Chromatographic techniques; Mass spectrometry

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1. Introduction

Pharmaceuticals are special commodities that maintain human health, and their quality control is paramount. High-quality drugs ensure patients receive the expected therapeutic effect, aiding in recovery and pain relief. Poor-quality drugs may render treatment ineffective, delaying patient recovery and increasing suffering and economic burden. In severe cases, they can cause serious adverse reactions, even life-threatening situations such as anaphylactic shock due to excessive impurities. Drug quality control also impacts the medical system's credibility and effectiveness. High-quality drugs enhance patient trust in the medical system, promote the healthy development of the pharmaceutical industry, protect people's health rights, and serve as the cornerstone of a solid public health

system.

2. The critical role of impurity analysis in drug testing

2.1. Identification and control of harmful impurities

In drug testing, impurity analysis is a key method for identifying and controlling harmful impurities. Through advanced analytical techniques like high-performance liquid chromatography, gas chromatography, and mass spectrometry, various impurities in drugs, including organic impurities, inorganic impurities, and residual solvents, can be precisely detected. Once harmful impurities are identified, corresponding control measures can be formulated based on their properties and sources. Starting from the source, strict control of raw material quality, optimization of the production process, and reduction of impurity generation are essential ^[1]. Monitoring should be strengthened during the production and storage processes to prevent impurity levels from exceeding standards. For harmful impurities already produced, methods such as purification and refinement can be employed to ensure impurity content in drugs remains within safe limits, guaranteeing drug safety and preventing adverse reactions and potential safety hazards for patients, allowing them to use medications safely.

2.2. Ensuring drug purity and stability

Drug purity and stability are important indicators of its quality, and impurity analysis plays a crucial role in this regard. The presence of impurities can directly affect the purity of drugs, reducing the content of active ingredients and affecting the treatment effect. Impurity analysis can accurately determine the types and content of impurities in drugs, ensuring that the purity of the drugs meets the standard requirements. In terms of stability research, impurity analysis can monitor the generation of impurities under various conditions such as temperature, humidity, and light. If an increase in impurities is detected, it indicates poor drug stability, and adjustments to the production process, packaging, or storage conditions may be necessary to inhibit the generation of impurities. Impurity analysis can also help researchers understand the degradation pathways and mechanisms of impurities, providing a scientific basis for drug stability and ensuring that drugs maintain stable quality during their shelf life, allowing patients to obtain reliable treatment effects.

2.3. Ensuring drug safety and effectiveness

The impact of drug impurity analysis on safety and effectiveness differs significantly between chemical drugs and biological products. In chemical drugs, even trace amounts of genotoxic impurities (such as nitrosamines) can cause DNA damage and pose a carcinogenic risk. Heavy metal residues (such as lead and arsenic) can accumulate in the body, posing a threat to kidney and liver safety. Residual solvents (such as toluene) may change the crystal form of the drug, affecting its dissolution rate and bioavailability. The core risks of biological products lie in process-related impurities: host cell proteins may trigger an immune response, neutralizing drug activity; nucleic acid residues have potential tumorigenicity; and misfolded protein aggregates not only reduce efficacy but may also enhance immunogenicity. Regarding effectiveness, degradation impurities in chemical drugs (such as oxidation products) may compete with the API for metabolic enzymes, while glycosylation variants in biological products may alter pharmacokinetic properties. Modern analytical techniques such as LC-MS/MS and CE-SDS can accurately identify different impurities. Through the QbD concept, control strategies can be established to achieve ppm-level control of

chemical drug impurities and ng/mg-level monitoring of host proteins in biological products, balancing safety thresholds and therapeutic activity.

3. Current status of impurity analysis techniques and methods

3.1. Application of chromatographic techniques in impurity analysis

Chromatographic techniques achieve separation based on differences in the distribution coefficients of different substances between the mobile and stationary phases. Ion chromatography, with its unique ion exchange principle, has been widely used in detection in fields such as food, environment, and biomedicine. Compared to traditional chromatography, ion chromatography exhibits significant advantages in detecting hydrophilic components such as organic acids and bases, and is also suitable for detecting complex organic molecules such as sugars and amino acids. Therefore, ion chromatography is often used for the inspection of active ingredients in complex drug raw materials or pharmaceutical preparations [2]. In drug impurity analysis, ion chromatography is mainly used to detect inorganic ions, organic acids, and their salts; gas chromatography is adept at analyzing volatile organic substances such as pesticide residues; and high-performance liquid chromatography, with its high resolution, sensitivity, and automation, is widely used to separate complex mixtures. However, chromatographic techniques also have limitations, such as complex sample pretreatment and high detection costs. Ion chromatography and gas chromatography play important roles in specific fields, while high-performance liquid chromatography, with its multi-mode separation capability, occupies an important position in the field of drug impurity analysis.

3.2. Application of mass spectrometry in impurity analysis

Mass spectrometry, which analyzes substances by measuring the mass-to-charge ratio (m/z) of molecular or atomic ions, plays a central role in the identification of pharmaceutical impurities. High-resolution mass spectrometry (HRMS), utilizing time-of-flight or Orbitrap analyzers, offers a resolution exceeding 100,000 and a mass accuracy below 1 ppm. This enables precise differentiation of impurities with similar molecular weights, such as isomers or homologues. For instance, in detecting nitrosamine genotoxic impurities in valsartan, HRMS achieves accurate qualitative and quantitative analysis at the ng/g level through precise mass matching and isotope abundance ratio analysis, overcoming the limitations of traditional mass spectrometry in identifying trace impurities. Combined with liquid/gas chromatography, HRMS not only resolves UV-nonresponsive impurities (e.g., N-nitrosodimethylamine) but also derives unknown impurity structures through multistage fragment ion analysis, significantly enhancing the reliability of trace impurity identification in complex matrices and providing a higher-dimensional technical guarantee for pharmaceutical impurity profiling control [3].

3.3. Other commonly used impurity analysis techniques

Techniques such as high-performance liquid chromatography and gas chromatography are also crucial in impurity analysis. High-performance liquid chromatography, based on the interaction between compounds in solution and the stationary and mobile phases, enables the separation, identification, and quantitative analysis of chemical components in pharmaceuticals, making it suitable for analyzing complex mixtures. Gas chromatography, with gas as the mobile phase, is primarily used for detecting pesticide residues like organochlorines. UV-visible spectrophotometry measures the absorbance of a drug at a specific wavelength, allowing for quantitative and qualitative analysis, and is

often used to determine the maximum absorption wavelength or to identify drugs through UV spectra.

4. Challenges and limitations of impurity analysis techniques

4.1. Improving analytical sensitivity and accuracy

In pharmaceutical impurity analysis, enhancing sensitivity and accuracy remains a pressing issue. On one hand, some impurities exist at extremely low levels, such as genotoxic impurities, which may be present at parts per billion concentrations, posing significant challenges to detection techniques [4]. Existing techniques like conventional high-performance liquid chromatography may not achieve the required sensitivity when detecting these impurities. On the other hand, the complexity of pharmaceutical components and severe matrix interference can also affect analytical accuracy. In complex sample analysis, two-dimensional chromatography (GC×GC or LC×LC) greatly improves resolution through orthogonal separation, but it suffers from high instrument costs and complex maintenance. Online purification techniques (e.g., online SPE) can reduce matrix interference with lower equipment investment but require optimization of purification steps. Supercritical fluid chromatography (SFC) offers high separation efficiency and low operating costs for non-polar impurities but has limited applicability. In contrast, conventional chromatography-mass spectrometry techniques (e.g., LC-MS) combine separation with high sensitivity, resulting in a higher overall cost (equipment + maintenance) but superior throughput and data reliability. Additionally, to balance economy and resolution, online purification combined with conventional chromatography or SFC can serve as an intermediate solution, while high-precision studies prioritize two-dimensional chromatography or hyphenated techniques [5]. Developing new analytical methods and technologies, such as hyphenated techniques that combine the advantages of multiple technologies like liquid chromatography-mass spectrometry, can also enhance the sensitivity and accuracy of impurity analysis in complex samples.

4.2. Difficulties in impurity analysis of complex samples

The analysis of impurities in complex samples faces numerous challenges. In complex systems such as traditional Chinese medicines (TCM) and biological products, the variety of components is extensive, and the types and sources of impurities are more intricate. Impurities in TCM may originate from various stages such as cultivation, processing, and storage of medicinal herbs, including pesticide residues, heavy metals, and mycotoxins. Biological products, on the other hand, may contain impurities like host cell proteins and nucleic acids. The physicochemical similarity between these impurities and the active ingredients of the drug increases the difficulty of separation and identification. Additionally, the matrix effect of complex samples cannot be ignored. Large molecules, ions, and other components in the matrix can interfere with instrument detection, leading to signal suppression or enhancement and affecting the accuracy of impurity quantification. For instance, in biological products, high concentrations of proteins may adsorb onto chromatographic columns, affecting their separation performance and, consequently, the results of impurity analysis. To address these issues, two-dimensional chromatographic techniques can be employed to enhance peak capacity and resolution. For example, in the impurity analysis of teicoplanin, 2D-UPLC-Q/TOF-MS is used in combination with desalting treatment after one-dimensional chromatographic separation to determine the structure of impurities [6]. Alternatively, supercritical fluid chromatography, which offers significantly higher efficiency compared to traditional liquid chromatography, can be utilized. For instance, in the separation of flavonoids from *Astragalus membranaceus*, a supercritical CO₂-methanol mobile phase is employed, and optimizing the modifier ratio (such as 13.64% entrainer) can improve the purity of paeoniflorin and albiflorin (94.11% and

85.65%, respectively) [7].

4.3. Issues with method reproducibility

Impurity analysis methods face reproducibility challenges. Variations in results can occur across different laboratories, operators, instruments, and even when using the same instrument at different times for the same impurity analysis. These discrepancies may be attributed to factors such as instrument stability, operational consistency, and sample uniformity. For example, in high-performance liquid chromatography (HPLC) analysis, batch differences in chromatographic columns, preparation errors in the mobile phase, and inaccuracies in sample injection volumes can all lead to variations in impurity peak areas or retention times, affecting method reproducibility. Similarly, in mass spectrometry, factors like instrument calibration status, ion source temperature, and mass spectrometry parameter settings can influence the stability of detection results. To enhance method reproducibility, it is essential to establish standardized operating procedures that strictly regulate instrument usage, maintenance, and calibration, ensuring instrument stability and consistency. Additionally, training laboratory personnel to improve their operational skills and adherence to protocols is crucial [8]. The use of reference materials for quality control, comparison, and calibration of analysis results can further ensure consistency across different laboratories and time points.

5. Effective paths for optimizing impurity analysis

5.1. Introducing advanced technological means

Ultra-high-performance liquid chromatography (UHPLC) technology has significantly accelerated analysis speed due to its use of smaller particle packing materials. For complex samples, this technology can accurately complete impurity analysis in a shorter time, representing a qualitative leap in analysis efficiency compared to traditional methods. The combination of chromatography and mass spectrometry, particularly liquid chromatography-mass spectrometry (LC-MS), is an innovative approach that seamlessly integrates the efficient separation capabilities of chromatography with the precise identification strengths of mass spectrometry. In the analysis of drug metabolites, for instance, LC-MS allows for the simultaneous and efficient detection of multiple components, greatly enhancing the comprehensiveness and accuracy of the analysis. On the other hand, gas chromatography-mass spectrometry (GC-MS) excels in pesticide residue detection, demonstrating high sensitivity and specificity in accurately identifying trace pesticide residues. These advanced technologies, with their outstanding characteristics, effectively overcome the challenges of impurity analysis in complex samples, providing solid and reliable data support for drug quality control and ultimately ensuring the safety and effectiveness of drugs in a comprehensive manner.

5.2. Establishing standardized work processes

In drug impurity analysis, standardized processes should span both intermediate and final product control, forming a closed-loop quality control system. Intermediate control involves setting standardized detection points at critical production nodes (such as before concentration of traditional Chinese medicine extracts or after purification steps in biological products). This includes specifying sampling methods, testing items (e.g., residual solvents or host protein content in intermediates), and thresholds. For example, HPLC can be used for rapid screening of pesticide residues in traditional Chinese medicine intermediates, while ELISA can dynamically monitor host DNA in biological fermentation broths, allowing for real-time interception of batches exceeding limits. Final product control, based on

regulations such as ICH Q3, involves establishing standardized testing procedures that cover both known and unknown impurities. For instance, LC-MS/MS combined with toxicity databases can be employed for impurity profiling in finished traditional Chinese medicines, while two-dimensional chromatography coupled with high-resolution mass spectrometry can identify trace amounts of host proteins in biological products. Concurrently, mandatory stability studies (including accelerated and long-term testing) should be conducted to monitor impurity growth trends during storage. Lastly, standardized analytical methods (such as unified chromatographic column models and mass spectrometry parameters) should be shared across both intermediate and final product control stages, with the adoption of automated equipment and information systems (LIMS) to ensure seamless data integration and minimize the risk of human intervention. Through this “process interception + terminal verification” strategy, lifecycle management of impurity risks can be achieved.

5.3. Strengthening personnel training and skill improvement

Personnel training is a core element in improving the quality of pharmaceutical impurity analysis. The professional knowledge and comprehensive abilities of analysts directly affect the accuracy and credibility of test results. Systematic training can help analysts quickly master cutting-edge detection techniques and methods, such as the operational skills and maintenance points of equipment like ultra-high-performance liquid chromatography and mass spectrometry, enabling them to skillfully use advanced tools for work. The training content should also comprehensively cover knowledge about drug impurities, including their sources, properties, and mechanisms affecting drug quality, to reinforce theoretical foundations. Additionally, practical operation training should be intensified to enhance analysts' problem-solving abilities through extensive hands-on experience. Furthermore, emphasis should be placed on cultivating a rigorous scientific attitude and a strong sense of responsibility, ensuring that drug impurity analysis results are accurate and effective from all dimensions, and providing solid human support for drug quality control.

5.4. Utilizing artificial intelligence and automation technology

Artificial intelligence and automation technology have immense potential in the field of pharmaceutical impurity analysis. Automation technology enables processes such as sample pretreatment, instrument operation, and data collection to be automated, significantly reducing errors caused by manual operations and enhancing analysis efficiency. For instance, automated equipment can accurately perform sample extraction, filtration, and other pretreatment steps, and can also control instruments according to preset programs, ensuring the stability of the analytical process. Artificial intelligence, leveraging machine learning algorithms, deeply mines massive amounts of impurity analysis data to discern potential patterns and trends, aiding in determining the source and properties of impurities. For example, AI combined with spectral analysis and deep learning can perform high-precision authenticity identification of medicinal materials like goji berries and *Cordyceps*, while tracing their origin (such as ginseng mainly produced in Northeast China) ^[9]. Additionally, AI plays a key role in data quality control, automatically screening abnormal data, further enhancing the accuracy and reliability of data analysis. For instance, through deep learning analysis of drug-target interactions, potential antiviral drugs (such as antiviral components in traditional Chinese medicine compounds) ^[10] can be quickly screened. The integration of these technologies creates smarter and more efficient technical solutions for drug quality control.

6. Conclusion

Impurity analysis in drug testing plays a crucial role in quality control. It identifies and controls harmful impurities, ensuring drug purity, stability, safety, and effectiveness. Among current impurity analysis techniques and methods, chromatographic and mass spectrometry techniques have their applications, but challenges such as improving analytical sensitivity and accuracy, addressing complexities in impurity analysis of complex samples, and method reproducibility still exist. In the future, drug impurity analysis needs continuous optimization. On one hand, advanced technologies like ultra-high-performance liquid chromatography and chromatography-mass spectrometry should be introduced to enhance analytical efficiency and accuracy. On the other hand, standardized work processes should be established, personnel training should be strengthened, and artificial intelligence and automation technology should be utilized to improve analysis quality and efficiency. It is believed that with advancing technology and refined methods, drug impurity analysis will play a greater role in drug quality control, providing stronger support for ensuring public medication safety and promoting the healthy development of the pharmaceutical industry.

Disclosure statement

The authors declare no conflict of interest.

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Innovative Technologies and Quality Evaluation of Oral Sustained-Release and Controlled-Release Preparations

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Abstract: This paper systematically explores the innovative technologies and quality evaluation system of oral sustained-release and controlled-release preparations. By combing the principles and limitations of traditional preparation technologies, it focuses on expounding novel innovative technologies such as ion-exchange resin, microsphere, and 3D printing, along with their application examples, and analyzes the advantages and disadvantages of each technology in drug release control. Meanwhile, it elaborates on the principles and applications of quality evaluation methods, including in vitro release rate test, in vivo bioavailability and bioequivalence evaluation, and in vitro-in vivo correlation evaluation, and illustrates the importance of quality evaluation in the research, development, and quality control of preparations through specific cases. In addition, it discusses the impact of innovative technologies on quality evaluation and proposes strategies for balancing innovation and quality control. The research shows that innovative technologies drive the development of oral sustained-release and controlled-release preparations, and a scientific quality evaluation system is crucial for ensuring the safety and effectiveness of preparations. The coordinated development of these two aspects helps to enhance the clinical application value of preparations.

Keywords: Oral sustained-release and controlled-release preparations; Innovative technologies; Quality evaluation; Drug release; Bioequivalence

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1. Introduction

In the field of modern medicine, oral administration, as the most common method of drug administration, is convenient and has high patient compliance. However, common oral preparations have problems such as large fluctuations in blood drug concentration, frequent medication administration, and low drug utilization rate. Oral sustained-release and controlled-release preparations, through special preparation technologies, can control the release rate and time of drugs in the body, maintain the blood drug concentration within the effective therapeutic

concentration range for a long time, reduce the frequency of medication administration, lower the toxic and side effects of drugs, and improve patient compliance and therapeutic effects^[1]. With the continuous development of medical technologies, innovative technologies for oral sustained-release and controlled-release preparations are constantly emerging. At the same time, a scientific and reasonable quality evaluation system is essential to ensure the safety, effectiveness, and quality stability of preparations. In-depth research on the innovative technologies and quality evaluation of oral sustained-release and controlled-release preparations is of great significance for promoting the development of this field and improving the level of drug treatment.

2. Innovative technologies of oral sustained-release and controlled-release preparations

2.1. Traditional preparation technologies

2.1.1. Technologies for controlling dissolution rate

Technologies for controlling the dissolution rate aim to delay the dissolution rate of drugs in the gastrointestinal tract by methods such as making drugs into salts or esters with low solubility, forming insoluble salts with macromolecular compounds, and controlling the particle size of drugs. For example, penicillin is made into procaine penicillin, which has reduced solubility and is slowly released in the body, prolonging the action time of the drug. However, this technology has certain limitations. The release rate of drugs is greatly affected by the gastrointestinal environment (such as pH value and enzyme activity), which may lead to unstable drug release^[2].

2.1.2. Technologies for controlling diffusion process

Technologies for controlling the diffusion process mainly include two types: reservoir-type and matrix-type. In reservoir-type preparations, drugs are wrapped in a polymer membrane, and the drugs are released into the surrounding medium through the diffusion of the membrane. In matrix-type preparations, drugs are dispersed in a polymer matrix material, and the drugs are released through the pores of the matrix. This technology can control the release rate of drugs to a certain extent. However, it is difficult to precisely control the drug release, and the degradation of the matrix material may affect the stability of drug release.

2.1.3. Technologies for coordinating diffusion and dissolution

Technologies for coordinating diffusion and dissolution usually involve wrapping the drug core with a film containing soluble pore-forming materials. When the drug is released, the water in the medium penetrates into the film, dissolves the pore-forming materials to form pores, and the drug is released through the pores. Meanwhile, the dissolution of the film also affects the drug release rate. Although this technology can achieve sustained and controlled drug release to a certain extent, the preparation process is relatively complex, and the performance requirements for materials are high. In practical applications, there are problems such as high costs^[3].

2.2. Novel innovative technologies

2.2.1. Ion-exchange resin technology

Ion-exchange resins are a class of polymer compounds with ion-exchange functions. In oral sustained-release and controlled-release preparations, drugs form drug-resin complexes with ion-exchange resins through ionic bonds. In

the gastrointestinal tract, the complexes undergo ion-exchange reactions with the ions in the gastrointestinal tract to release the drugs. For example, methylphenidate hydrochloride binds to ion-exchange resins and exchanges with sodium ions in the gastrointestinal tract to achieve slow drug release [4]. This technology has advantages such as less influence of the gastrointestinal pH value on drug release and the ability to prepare liquid formulations, which are convenient for children and elderly patients. However, it has limitations such as limited drug loading capacity and slow release rate.

2.2.2. Microsphere technology

Microsphere technology involves dissolving or dispersing drugs in polymer materials to prepare spherical particles with a particle size of 1- 250 μ m. The main preparation methods of microspheres include emulsification-solidification method and spray drying method. Taking leuprolide microspheres as an example, leuprolide is mixed with biodegradable polymer materials (such as poly (lactic-co-glycolic acid)) and prepared into microspheres through the emulsification-solidification method. After the microspheres enter the body, the drugs are slowly released with the degradation of the polymer materials, achieving long-acting sustained release. Microsphere technology can effectively control the drug release rate, reduce the frequency of drug administration, and improve drug stability. However, it has problems such as complex preparation processes and high costs [5].

2.2.3. 3D printing technology

The application of 3D printing technology in oral sustained-release and controlled-release preparations has become a research hotspot in recent years. Through computer-aided design (CAD) software, the three-dimensional structure of the preparation is designed, and then the drug and polymer materials are printed layer by layer using a 3D printer according to the design model. This technology can precisely control parameters such as the shape, size, and porosity of the preparation according to the characteristics of the drug and the individual needs of patients, achieving personalized customization and precise drug release. For example, multi-layer structures with different drug release rates can be prepared by adjusting the printing parameters. However, the application of 3D printing technology in oral preparations currently faces challenges such as limited printing materials, low production efficiency, and difficult quality control.

3. Quality evaluation of oral sustained-release and controlled-release preparations

3.1. Importance of quality evaluation

The quality evaluation of oral sustained-release and controlled-release preparations is a crucial link in ensuring the safety, effectiveness, and quality stability of preparations. A scientific and reasonable quality evaluation can ensure the consistency of preparations among different batches, ensure that the drug release behavior in the body meets expectations, and thus guarantee the safety of patient medication and therapeutic effects [6]. At the same time, quality evaluation is also an important basis in the preparation research and development process, which helps to optimize the formulation and process of preparations and improve the quality and performance of preparations.

3.2. Quality evaluation methods

3.2.1. In vitro release rate test

The in vitro release rate refers to the rate and extent of drug release from sustained-release and controlled-release

preparations, which is one of the important indicators for evaluating the quality of oral sustained-release and controlled-release preparations. Commonly used release rate determination methods include the basket method, paddle method, and small cup method. When selecting the determination method, factors such as the dosage form of the preparation and the drug release mechanism need to be comprehensively considered. The selection of the release medium is also of great importance. Generally, aqueous media such as 0.1mol/L hydrochloric acid solution and pH 6.8 phosphate buffer solution are selected to simulate the gastrointestinal environment [7]. In addition, factors such as the pH value, ionic strength, and surface tension of the medium that affect drug release need to be considered. The rotation speed and instrument device also affect the drug release behavior, and reasonable selection should be made according to the characteristics of the preparation.

3.2.2. In vivo bioavailability and bioequivalence evaluation

Bioavailability refers to the rate and extent of drug absorption into the bloodstream after extravascular administration. For oral sustained-release and controlled-release preparations, bioavailability evaluation can be carried out by measuring the blood drug concentration-time curve and calculating parameters such as the area under the curve (AUC), time to peak concentration (T_{max}), and peak concentration (C_{max}). Bioequivalence means that different formulations of a drug have no significant differences in absorption rate and extent under the same test conditions when given the same dose. In the research and development of oral sustained-release and controlled-release preparations, bioequivalence tests are usually carried out, and the test preparation is compared with the reference preparation to determine whether they are bioequivalent. Bioequivalence evaluation is an important means to ensure the clinical effectiveness and safety of preparations.

3.2.3. In vitro-in vivo correlation evaluation

In vitro-in vivo correlation refers to the correlation between the in vitro release behavior of preparations and the pharmacokinetic parameters in vivo. Establishing in vitro-in vivo correlation can predict the drug release behavior in vivo through in vitro release rate tests, to optimize the formulation and process of preparations and reduce the number of in vivo tests. In vitro-in vivo correlation mainly includes three types: A, B, and C. Type A correlation is the most ideal, which reflects the point-to-point relationship between the in vitro release time and the in vivo pharmacokinetic curve. Type B correlation is the relationship between the in vitro average release time and the in vivo mean residence time established through statistical moment analysis. Type C correlation is the single-point relationship between in vitro release parameters and in vivo pharmacokinetic parameters. In practical research, an appropriate type of in vitro-in vivo correlation should be selected according to the characteristics of the preparation for research.

3.3. Case analysis

Taking nifedipine sustained-release tablets as an example, in the in vitro release rate test, the paddle method is adopted, 0.1mol/L hydrochloric acid solution is used as the release medium, and the rotation speed is 50r/min. The drug release amount is determined by sampling at different time points. Through the analysis of the release curve, it is judged whether the drug release behavior of the preparation meets the design requirements. In the in vivo bioavailability and bioequivalence evaluation, healthy volunteers are selected for the test. The test preparation and the reference preparation are given respectively, the blood drug concentration-time curve is measured, and relevant pharmacokinetic parameters are calculated. The results show that there are no significant differences in parameters

such as AUC, Cmax, and Tmax between the test preparation and the reference preparation, indicating that they are bioequivalent. In the in vitro-in vivo correlation evaluation, by establishing the relationship between the in vitro release rate and the in vivo pharmacokinetic parameters, it is verified that the preparation has a good in vitro-in vivo correlation, providing strong support for the quality control and clinical application of the preparation.

4. The relationship between innovative technologies and quality evaluation

4.1. Impact of innovative technologies on quality evaluation

The application of novel innovative technologies has changed the drug release mechanism and release behavior of oral sustained-release and controlled-release preparations, and put forward new requirements for quality evaluation indicators and methods. For example, preparations prepared by 3D printing technology have unique three-dimensional structures, and their in vitro release behavior may be different from that of traditional preparations. Traditional release rate determination methods may not be able to accurately evaluate their release characteristics, and new evaluation methods and indicators need to be developed. For preparations prepared by ion-exchange resin technology, the drug release is affected by ion-exchange kinetics, and factors such as ion concentration and exchange rate need to be considered in quality evaluation.

4.2. Feedback of quality evaluation on innovative technologies

Quality evaluation results can provide important bases for the optimization and improvement of innovative technologies. Through the analysis of preparation quality evaluation data, problems in the application process of innovative technologies, such as unstable drug release and low bioavailability, can be found. In response to these problems, R&D personnel can adjust and optimize innovative technologies, improve the formulation and process of preparations, and enhance preparation quality. For example, in the application of microsphere technology, if the quality evaluation finds that the drug release rate of microspheres does not meet expectations, it can be optimized by adjusting the composition of polymer materials and the preparation process of microspheres [8].

4.3. Strategies for coordinated development

To achieve the coordinated development of innovative technologies and quality evaluation for oral sustained-release and controlled-release preparations, it is necessary to strengthen interdisciplinary cooperation and promote the cross-integration of disciplines such as pharmacy, materials science, and analytical chemistry [9]. In the research and development process of innovative technologies, the requirements of quality evaluation should be fully considered, and the concept of quality evaluation should be implemented throughout the preparation research and development process. At the same time, quality evaluation methods should be continuously innovated and improved to meet the development needs of novel innovative technologies. In addition, relevant laws, regulations, and standards should be established and improved to standardize the application of innovative technologies and quality evaluation behaviors, and ensure the quality and safety of oral sustained-release and controlled-release preparations [10].

5. Conclusion

This paper systematically studies the innovative technologies and quality evaluation of oral sustained-release and controlled-release preparations. In terms of innovative technologies, traditional preparation technologies have certain limitations, while novel innovative technologies such as ion-exchange resin, microsphere, and 3D printing show

unique advantages in drug release control, but they also face their own challenges. In terms of quality evaluation, methods such as in vitro release rate test, in vivo bioavailability and bioequivalence evaluation, and in vitro-in vivo correlation evaluation complement each other, forming a quality evaluation system for oral sustained-release and controlled-release preparations, which is crucial for ensuring preparation quality and clinical effectiveness. At the same time, innovative technologies and quality evaluation influence and promote each other, and their coordinated development helps to improve the overall level of oral sustained-release and controlled-release preparations.

Disclosure statement

The author declares no conflict of interest.

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The Efficacy of Jinlida Granules on Type 2 Diabetes Mellitus was Discussed Based on the Theory of “Treating from Spleen”

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Abstract: *Objective:* To explore the clinical efficacy of the spleen-strengthening and body fluid-moving formula, Jinlida Granules, in combination with Metformin for treating type 2 diabetes based on the theory of ‘treating from the spleen.’ *Methods:* A case-control study was conducted, involving 62 patients with type 2 diabetes admitted between August 2022 and December 2024. These patients were randomly divided into a control group (31 patients treated with Metformin) and an observation group (31 patients treated with Jinlida Granules), both for a 12-week course of treatment. The study compared changes in blood glucose levels (FPG, 2hPG, HbA1c), lipid levels (TC, TG, LDL, HDL), and pancreatic function indicators (HOMA-B, HOMA-IR) before and after treatment in both groups, and assessed the overall clinical effectiveness. *Results:* The overall effectiveness rate of the observation group was 90.3%, significantly higher than the 74.2% in the control group ($P < 0.05$). After treatment, the FPG in the observation group decreased by $2.1 \pm 0.8\%$ mmol/L, and HbA1c decreased by $1.5 \pm 0.6\%$. Additionally, the 2hPG in the observation group was significantly better than that in the control group, with a $18.3 \pm 5.2\%$ increase in HOMA-B and a $1.5 \pm 0.6\%$ decrease in HOMA-IR, all of which were better than those in the control group ($P < 0.05$). *Conclusion:* Jinlida Granules combined with Metformin can significantly improve the glucose and lipid metabolism and pancreatic cell function in patients with type 2 diabetes.

Keywords: Treating from the spleen; Jinlida granules; Type 2 diabetes

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1. Introduction

In traditional Chinese medicine (TCM), the spleen is often regarded as the pivotal organ for the body's Qi movement. Its ability to transform and transport nutrients and fluids directly affects the body's metabolic balance. The “Huangdi Neijing” (Yellow Emperor's Inner Canon) states, “When food enters the stomach, it circulates with vital energy, which then ascends to the spleen, where the spleen disperses the essence.” If this dynamic regulatory system,

centered on the spleen, malfunctions, it can lead to the pathological condition of “water turning into dampness and grains becoming stagnant” [1]. In modern medicine, the typical symptoms of diabetes, such as polyuria, polydipsia, polyphagia, and weight loss, closely mirror the TCM concept of “spleen deficiency leading to impaired transformation and distribution of body fluids,” which results in diabetes [2]. When the spleen fails to function properly, it not only prevents the transformation of nutrients and fluids into Qi and blood but also leads to the accumulation of turbid substances [2]. In this pathological state, the abnormal rise in blood sugar is merely an external manifestation of organ dysfunction, with the core issue being the spleen’s failure to properly transform and distribute body fluids, leading to a comprehensive disorder in the metabolism of qi, blood, and body fluids [3]. From a clinical perspective [4], while the traditional method of “nourishing yin and clearing heat” can alleviate some symptoms of diabetes, it often overlooks the spleen’s crucial role in the distribution of body fluids, leading to a situation where treating the symptoms is difficult without addressing the root cause. For example, some patients who take cold-natured medications for a long time may experience reduced appetite and heaviness in the limbs, indicating damage to the spleen yang. This phenomenon has prompted medical practitioners to reassess the theoretical value of “treating from the perspective of the spleen,” emphasizing that “strengthening the spleen and transforming body fluids” is not just about tonifying the spleen qi but also about restoring the spleen’s physiological functions of transforming and distributing body fluids to the lungs [5]. By activating the spleen’s ability to transport the essence of food and water, it reconstructs the metabolic cycle where the spleen disperses essence, which then returns to the lungs, regulates the water pathways, and transports it to the bladder. This holistic regulatory approach resonates with modern medicine’s goals of improving insulin resistance and repairing pancreatic function. The formula of Jinlida Granules, with its key ingredients like Astragalus and Atractylodes for strengthening the spleen and drying dampness, is complemented by Pueraria root to enhance spleen yang and Coptis to clear heat, embodying the principle of ‘pungent to open, bitter to descend.’ This not only restores the Qi mechanism in the middle energizer but also promotes the normal distribution of body fluids [6,7]. This study, based on the theory of ‘treating from the spleen,’ explores the clinical efficacy of the spleen-strengthening and body fluid-regulating formula Jinlida Granules combined with Metformin in treating type 2 diabetes.

2. General information and methods

2.1. General information

A case-control study design was conducted, involving 62 patients with type 2 diabetes admitted between August 2022 and December 2024. The patients were randomly divided into a control group (31 cases treated with metformin) and an observation group (31 cases treated with Jinlida Granules). The 31 patients in the control group ranged in age from 47 to 70 years, with an average age of 55.1 ± 7.0 years old. Among them, 15 were male (48.4%) and 16 were female (51.6%), with an average BMI of 25.9 ± 3.1 kg/m². The 31 patients in the observation group ranged in age from 45 to 68 years, with an average age of 54.2 ± 6.3 years old. Of these, 17 were male (54.8%) and 14 were female (45.2%), with an average BMI of 26.3 ± 2.8 kg/m².

Inclusion criteria: (1) Conforming to the World Health Organization (WHO) diagnostic criteria for type 2 diabetes mellitus; (2) No insulin treatment within the past 6 months; (3) Fasting blood glucose > 7.0 mmol/L and postprandial 2-hour blood glucose ≥ 11.1 mmol/L; (4) Age 40–70 years old.

Exclusion criteria: (1) Severe organ dysfunction, such as renal failure or decompensated liver cirrhosis; (2) History of immune system diseases, mental disorders, or malignant tumors; (3) Known allergy to metformin or the components of Jinlida Granules; (4) Pregnant or breastfeeding women; (5) Women who have participated in clinical

trials of other antidiabetic drugs within the past 3 months.

2.2. Method

2.2.1. Drug regimen of the control group

Take the oral metformin hydrochloride tablets (0.5 g per tablet) at a starting dose of 500 mg twice daily with meals. Based on the patient's blood glucose levels and gastrointestinal tolerance, the dose should be increased by 500 mg weekly, with a maximum daily dose of 2000 mg. During treatment, monitor liver and kidney function as well as blood glucose levels. Adjust the current dose or make appropriate adjustments based on individual conditions as needed.

2.2.2. Medication regimen of the observation group

In addition to the medication in the control group, Jinlida Granules (ingredients: Astragalus, Atractylodes, Pueraria, Coptis, etc., specification: 9 g/bag) are taken at a dose of 9 g three times daily, 30 minutes before meals, mixed with 200 mL of warm water. The dosage adjustment principle for Metformin is the same as that of the control group. Jinlida Granules should be taken continuously for 12 weeks, during which other Chinese herbal hypoglycemic preparations are prohibited. Liver and kidney function should be rechecked every 4 weeks during treatment. If severe diarrhea or gastrointestinal discomfort occurs, Jinlida Granules should be suspended for 3 days, and the dose should be adjusted to 6 g per dose.

2.3. Evaluation criteria

2.3.1. Evaluation of clinical efficacy

According to the "Guidelines for the Prevention and Treatment of Type 2 Diabetes in China" and the improvement rate of TCM syndrome scores, the criteria are as follows: For significant effect, FPG should be ≤ 7.0 mmol/L, HbA1c should decrease by at least 1.5%, and the TCM syndrome score should decrease by at least 60%; for effective effect, FPG should decrease by at least 10% and HbA1c should decrease by at least 0.5%, and the TCM syndrome score should decrease by at least 30%; for ineffective effect, if the above criteria are not met, the total effective rate is calculated as (number of cases with significant effect + number of cases with effective effect) / total number of cases $\times 100\%$.

2.3.2. Evaluation of blood glucose metabolism

Fasting blood glucose (FPG) and 2-hour postprandial blood glucose (2hPG) were detected by a fully automatic biochemical analyzer, and hemoglobin A1c (HbA1c) was analyzed by high-pressure liquid phase method, and a difference of $\geq 10\%$ before and after treatment was considered as an effective improvement.

2.3.3. Evaluation of blood lipid metabolism

A decrease of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) by more than 10% or an increase of high-density lipoprotein cholesterol (HDL-C) by more than 0.1 mmol/L is considered as effective regulation. The detection is performed by fully automatic biochemical analyzer, and the operation is carried out according to the kit instructions.

2.3.4. Evaluation of islet function

The steady-state model evaluation method was used to calculate the pancreatic β -cell function index (HOMA-B) and insulin resistance index (HOMA-IR). The improvement of pancreatic function was determined by HOMA-B increased by more than 10% and HOMA-IR decreased by more than 0.5 after treatment.

2.4. Statistical methods

The data were imported into SPSS 26.0 software for statistical analysis, and the measurement data were presented in the form of mean \pm standard deviation (SD). The independent sample *t*-test was used for inter-group comparison, and the rate (%) was used to express the count data and the chi-square test was used. $P < 0.05$ was set as the threshold value for statistically significant difference.

3. Results

3.1. Comparison of total efficiency

The total effective rate of the observation group was 90.3%, significantly higher than that of the control group (74.2%) ($P < 0.05$) (Table 1).

Table 1. Comparison of total effective rate between the two groups

Group	Example	Efficacy (example)	Effective (example)	Ineffective cases(example)	Overall effective rate (%)
Observation group	31	15	13	3	90.3%
Control group	31	10	13	8	74.2%
χ^2					8.294
<i>P</i>					0.000

3.2. Blood sugar index

After treatment, the FPG of the observation group decreased (2.1 ± 0.8) mmol/L and HbA1c decreased (1.5 ± 0.6)%, and the 2hPG index of the patients in the observation group was significantly better than that of the control group, and the improvement degree was significantly better than that of the control group ($P < 0.05$) (Table 2).

Table 2. Changes of blood glucose indexes in two groups of patients (mean \pm SD)

Parameters	Group	Before treatment	After treatment
FPG (mmol/L)	Observation group	9.2 ± 1.5	7.1 ± 1.2
	Control group	9.3 ± 1.6	8.0 ± 1.3
HbA1c (%)	Observation group	8.5 ± 1.0	7.0 ± 0.8
	Control group	8.6 ± 1.1	7.6 ± 0.9
2hPG (mmol/L)	Observation group	12.5 ± 1.0	7.6 ± 0.8
	Control group	12.6 ± 1.1	9.1 ± 0.9

3.3. Lipid indicators

The degree of TC, TG, LDL reduction and HDL increase in the observation group was significantly higher than that in the control group ($P < 0.05$) (Table 3).

Table 3. Changes of lipid indexes in two groups of patients (mean \pm SD)

Parameters	Group	Before treatment	After treatment
TC (mmol/L)	Observation group	5.8 \pm 1.2	4.5 \pm 0.9
	Control group	5.7 \pm 1.1	5.0 \pm 1.0
TG (mmol/L)	Observation group	2.5 \pm 0.7	1.8 \pm 0.5
	Control group	2.6 \pm 0.6	2.2 \pm 0.6
LDL (mmol/L)	Observation group	3.4 \pm 0.8	2.6 \pm 0.6
	Control group	3.3 \pm 0.7	3.0 \pm 0.7
HDL (mmol/L)	Observation group	1.0 \pm 0.2	1.3 \pm 0.3
	Control group	1.1 \pm 0.2	1.2 \pm 0.2

3.4. Insulin function index

In the observation group, HOMA-B increased by 18.3 \pm 5.2% and HOMA-IR decreased by 1.5 \pm 0.6%, both significantly better than the control group ($P < 0.05$) (Table 4).

Table 4. Changes of pancreatic function indexes (mean \pm SD) in two groups of patients

Parameters	Group	Before treatment	After treatment
HOMA-B (%)	Observation group	60.0 \pm 10.0	78.3 \pm 12.0
	Control group	61.0 \pm 9.5	68.0 \pm 10.5
HOMA-IR	Observation group	4.5 \pm 1.2	3.0 \pm 0.8
	Control group	4.6 \pm 1.1	3.8 \pm 1.0

4. Discussion

Type 2 diabetes is characterized by insulin resistance and the progressive decline of β -cell function. The “high sugar toxicity” it causes often leads to metabolic disorders, including lipid abnormalities, chronic inflammation, and damage to multiple systems [8]. While modern medicine can control blood glucose fluctuations with short-term hypoglycemic drugs, it struggles to reverse the fundamental TCM pathogenesis of spleen dysfunction and fluid distribution disorders. As stated in the “Suwen Qibing Lun”: “This condition arises from overindulgence in rich foods; the person must frequently consume sweet and fatty foods. Fatty foods cause internal heat, while sweet foods lead to fullness in the middle, causing qi to rise and transform into thirst,” highlighting the pathogenic chain of spleen dysfunction caused by excessive consumption of rich and sweet foods [9]. The formula of Jinli Da Granules focuses on “restoring the spleen’s transformation and promoting the smooth flow of the middle energizer.” It uses Astragalus (a warming herb) to tonify Qi and elevate Yang to invigorate the spleen Yang, Atractylodes (a bitter-warm herb) to dry dampness and awaken the spleen to expel internal dampness, Pueraria (a raw herb) to generate body fluids and clear the clear, assisting the spleen to disperse essence, and Coptis (a bitter-cold herb) to clear and purge depressed heat and prevent dampness from transforming into fire. The combination of these four herbs, with their pungent and bitter properties, avoids the drawbacks of Yin-nourishing herbs that can be greasy and obstruct the stomach, as well as the risks of clearing heat herbs that can damage the middle yang, allowing body fluids to ‘ascend and descend’ and restore their normal flow [10].

In this study, the total effective rate of the observation group increased by 16.1% compared to the control group (90.3% vs 74.2%). The HbA1c level decreased by 1.5%, and the HOMA-B score increased by 18.3%. These results

confirm the unique advantages of the spleen-strengthening and fluid transporting method in improving insulin secretion function. The spleen's role in governing muscles and promoting metabolism means it plays a crucial role in regulating glucose uptake and transport. When the spleen is strong, nutrients are efficiently distributed to muscle tissue for oxidation and energy production rather than being retained in the blood as "turbid sugar." This aligns with modern medical theories on enhancing skeletal muscle insulin sensitivity and inhibiting liver glucose output. The active ingredients in Jinlida Granules, such as Puerarin and Astragaloside A, have been shown to activate the AMPK signaling pathway, promoting glycolipid metabolism, which is a molecular biological expression of the "spleen dispersing essence" theory ^[11]. The LDL level in the observation group decreased by 0.8 mmol/L, and the HDL level increased by 0.3 mmol/L, showing a better lipid-regulating effect compared to the control group. This improvement is due to the pathological characteristics of fat turbidity accumulation in the spleen deficiency and impaired transportation. According to "Medical Classics Must Read," "the change from rich food can lead to severe boils; in wealthy and noble people, this disease is caused by rich food." When the spleen fails to disperse essence, the fat does not return to its normal state, leading to stagnation in the pulse channels, which is known as phlegm and stasis. Jinlida Granules restore the spleen's ascending function, converting lipids into Qi, blood, and body fluids rather than depositing them as pathological products. Its multi-target regulation characteristic compensates for the limitations of Western medicine, which focuses on enzyme inhibitors ^[12]. The 18.3% improvement in pancreatic β -cell function suggests that the spleen-strengthening and fluid-moving method may delay the apoptosis of β -cells during the natural course of diabetes. This aligns well with the theory that "the spleen is the foundation of postnatal health and the source of qi and blood production."

In a state of spleen deficiency, oxidative stress and endoplasmic reticulum stress continuously attack β -cells. Components such as astragalus polysaccharides and atractylodes ketone can reduce pancreatic inflammation by inhibiting the NF- κ B pathway. This treatment strategy of "strengthening the middle energizer to nourish the innate constitution" provides a new paradigm for the integrated Chinese and Western medicine intervention in type 2 diabetes ^[13,14]. Current diabetes management is shifting from a "blood glucose-centric" approach to a "metabolic homeostasis holistic regulation." The concept of "spleen-body-fluid-metabolism" axis regulation embodied in Jinlida granules not only offers a modern interpretation of the "treatment from the spleen" theory but also highlights the importance of restoring the ascending and descending Qi mechanism of the middle energizer or breaking the vicious cycle of insulin resistance. Future research should further explore the mechanisms linking "spleen governing transformation and transportation" with the intestinal microbiota and adipokine network systems, promoting the deep integration of traditional Chinese medicine theory with precision medicine.

5. Conclusion

To sum up, the combination of Jinlida granules and Metformin can significantly improve the glucose metabolism and islet cell function of patients with type 2 diabetes, clarify the theoretical basis of "treating from the spleen" for the clinical guidance of type 2 diabetes, and provide new ideas for the treatment of diabetes.

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Disclosure statement

The author declares no conflict of interest.

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Determination of Isomatrine in Traditional Mongolian Medicine Agar-15 Powder by HPLC

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Abstract: A qualitative and quantitative method for the determination of flavonoids in traditional Mongolian medicine agar-15 powder was established. C18.6 mm chromatographic column is selected \times 250 mm, particle size 5 μ m. The mobile phase system is acetonitrile water, gradient elution. The detection wavelength was 295 nm. The range of isomatrine was 0.284–2.84 μ g. The regression equation is $y = 25.811x - 0.1969$, $r = 0.99990$. The recovery was 91.39% and RSD was 1.42%. All the methods mentioned in this paper have the characteristics of simplicity, accuracy, high sensitivity, good reproducibility and strong durability, which provide a reference for the quality control of agar-15 powder.

Keywords: Agar-15 powder; Isomatrine; High performance liquid chromatography; Content determination

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1. Introduction

Agar-15 powder is a traditional Mongolian medicine preparation widely used in Inner Mongolia. Its efficacy is to calm Qi, heat and viscosity [1]. It is mainly used for Qi and blood asthma, cough, late stage of fever, heart heat, heart Qi and blood headache. It is now included in the specification for Mongolian medicine preparations in Inner Mongolia (2007 Edition) (Volume I) [1]. The prescription of agar-15 powder is composed of fifteen herbs, based on *Sophora flavescens* function in clearing heat, drying and dampness, supplemented by fourteen other herbs. There are two main types of chemical components in *Sophora flavescens*, namely alkaloids and flavonoids, which are also the main pharmacologically active components of *Sophora flavescens*. In the current standard of agar-15 powder, only the alkaloid components of *Sophora flavescens* are identified, and the test items of flavonoids contained in *Sophora flavescens* are not involved at all, which cannot scientifically and objectively reflect the effectiveness of the drug. Therefore, the relevant test items of *Sophora flavescens* in agar-15 powder are supplemented [2].

2. Material

Thermofisher Ultimate 3000 high-performance liquid chromatograph, Waters e2695 high-performance liquid chromatograph, Satoris BSA224S electronic balance ($d = 0.1\text{mg}$), Satoris BP211D electronic balance ($d = 0.01\text{ mg}$), Kunshan ultrasonic instrument KQ500DE numerical control ultrasonic cleaning instrument.

Anthocyanin reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190214, purity: 98.06%), matrine reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190511, purity: 96.49%), isomatine reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190409, purity: 98.95%) Matrinol I reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 190513, purity: 99.74%), Gaolihuaisu reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 19070502, purity: 99.50%) and Clover bean red sandalwood glycoside reference substance (source: Beijing Putian Tongchuang Biotechnology Co., Ltd., batch number: 19080508, purity: 99.75%), five batches of agar-15 powder samples were from Hohhot Mongolian medicine hospital, Alashan League Mongolian medicine hospital, Xianghuang Banner Mongolian medicine hospital, Xilin Gol League Mongolian medicine hospital and Inner Mongolia International Mongolian medicine hospital [3].

3. Experimental methods and results

3.1. Chromatographic conditions and system suitability test

Chromatographic column: the filler is octadecyl silane-bonded silica gel. Omni Hubble C18 column (SN: 132235) (specification: 250) is used in this experiment $\times 4.6\text{mm}$, particle size $5\text{ }\mu\text{m}$) And thermofisher BDS hypersil C18 column (SN: 12185170jr4) (specification: $250 \times 4.6\text{ mm}$, particle size $5\text{ }\mu\text{m}$); Gradient elution with acetonitrile water as mobile phase, 0–25min, 40–50% a, 25–30 min, 50–70 A, 30–40 min, 70% a, 40–50min, 70–40% a; Detection wavelength: 295 nm; Flow rate: 1.0 mL/min; The injection volume of standard sample and test sample is the same as $10\text{ }\mu\text{L}$. Under the above chromatographic conditions, the theoretical plates calculated by the isomatine peak is not less than 4000, the resolution with adjacent component peaks is greater than 1.5, and the symmetry factor of chromatographic peaks is 0.595–1.05. Typical chromatograms are shown in **Figure 1–Figure 3**.

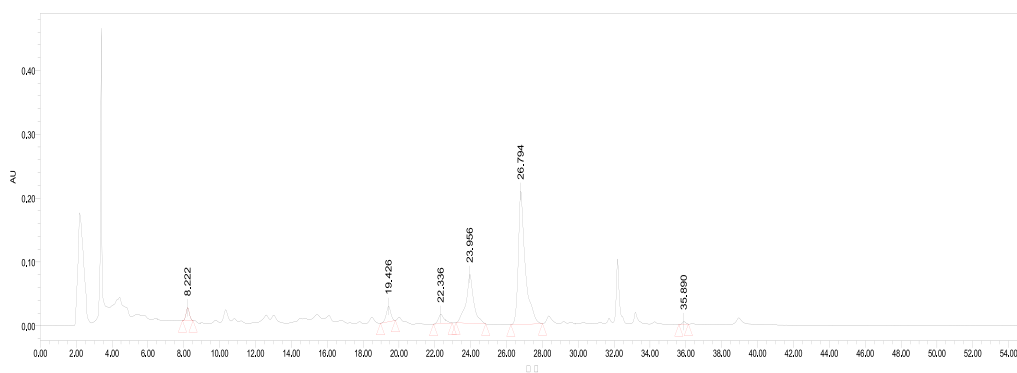


Figure 1. Chromatogram of mixed standard separation effect (the peak sequence is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaolihuaisu, matriol, matrine and isomatine).

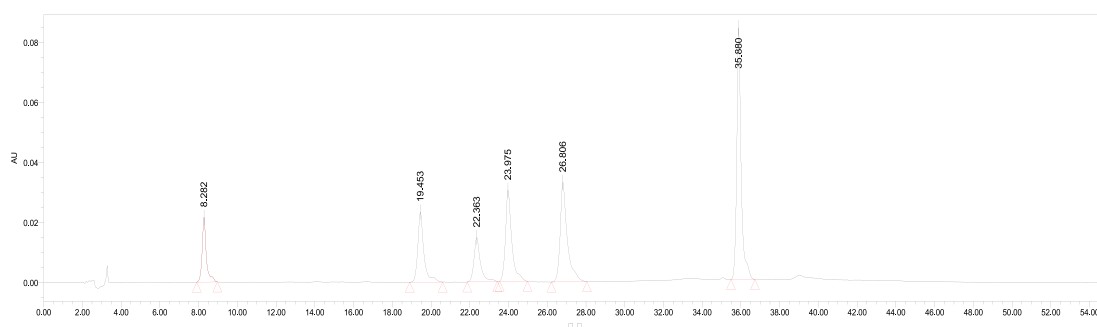


Figure 2. Chromatogram of separation effect of representative samples (the peak sequence is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaoliuhaisu, matriol, matrine and isomatrine).

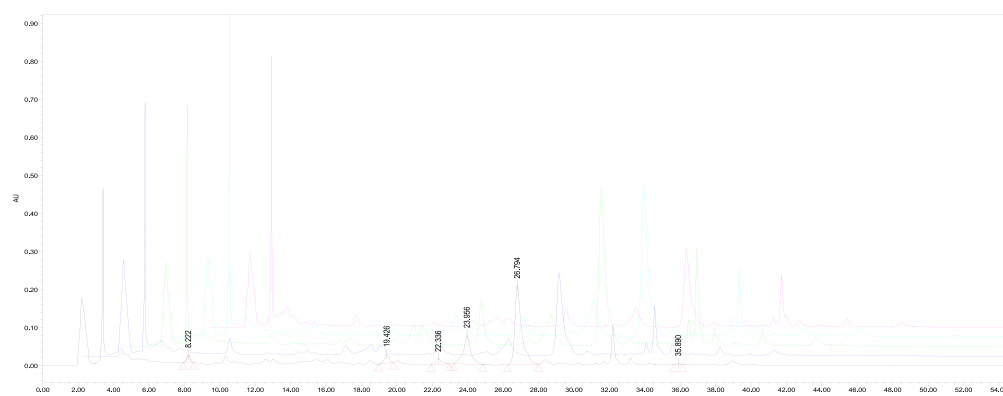


Figure 3. Chromatogram of separation effect of 5 batches of samples (the order of peaks is as follows: Sandalwood glycoside of clover bean, anthocyanin of prickly awn stalk, gaoliuhaisu, matriol, matrine and isomatrine).

3.2. Preparation of solution

3.2.1. Preparation of the reference solution

Take a proper amount of the reference substance of anthocyanin, matrine, isomatrine, matriol I, sophoricin and tripterygium rosewood glycoside, accurately weigh, and add methanol to prepare a solution containing 1 mg of isomatrine per 1 mL.

3.2.2. Preparation of test solution

Preparation of test solution: take about 11.0 g of powder (passing No. 3 sieve), weigh accurately, put it into a conical flask with a stopper, precisely add 20 mL of methanol, close the stopper, weigh the weight, conduct ultrasonic treatment (power 250 W, frequency 33 kHz) for 60 minutes, cool, weigh again, make up the lost weight with methanol, shake well, filter, and take the continuous filtrate [4].

3.2.3. Negative control test

Prepare the negative control without *Sophora flavescens* in strict accordance with the prescription of agar-15 powder, and prepare the negative control solution according to the preparation method of the test solution. Under the above chromatographic conditions, accurately absorb 10% of the control solution, 10% of the test solution and 10% of the negative control solution, respectively, and inject them into the liquid chromatograph. The measured results are as follows: in the negative control chromatogram, the colorless peak appears at the retention time corresponding to the

chromatograms of the above six controls and test samples, indicating that other drug components in the formulation do not interfere with the determination of the above six components [5].

3.3. Investigation of linear relationship

Precision absorption of isomatriline standard solution (the content of isomatriline is 284 µg/mL): 1 µL, 2 µL, 6 µL, 8 µL, 10 µL injected into the liquid chromatograph, measured according to the chromatographic conditions, and the peak area. The injection volume was analyzed by linear regression with peak area. The range of isomatriline was 0.284–2.84 µL. The regression equation is $y = 25.811x - 0.1969$, $r = 0.99990$.

3.4. Precision test

Take the same isomatriline reference solution (the content of isomatriline is 284 µg / mL), repeat the injection 6 times according to the chromatographic conditions under item 2.1, and the RSD of the peak area of isomatriline is 1.2%. The results show that the precision of the instrument is good [6].

3.5. Repeatability test

Take 6 samples of known isomatriline content in the same batch (66.16 µg / g), prepare the test solution according to the proposed method, determine the content of each sample, and calculate the relative standard deviation of the content value, as shown in **Table 1**.

Table 1. Repeatability of methods

Sampling quantity(g)	Isomatriline peak area	Isomatriline content	Average content of isomatriline	RSD(%)
11.0013	1.352	66.07µg/g	66.68µg/g	0.85
11.0516	1.419	66.03µg/g		
11.1029	1.499	66.51µg/g		
11.0094	1.381	66.95µg/g		
11.0533	1.451	67.41µg/g		
11.0125	1.388	67.08µg/g		

3.6. Sample adding recovery test

Take 5 known isomatriline contents of the same batch (66.16 µg / g), accurately weigh, and accurately add the spiked standard solution (the content of isomatriline is 28.4 µg / mL), prepare the test solution according to the proposed method, determine the content of each, and calculate the recovery (%), as shown in **Table 2**.

Table 2. Test results of standard addition and recovery of isomatriline

Sampling quantity (g)	Content of isomatriline in test sample (µg)	Addition amount of reference substance (µg)	Measured content (µg)	Rate of recovery (%)	Average recovery (%)	RSD (%)
11.0113	66.91	28.4	92.78	91.09	91.05	1.23
11.0024	66.32	28.4	91.89	90.07		
11.0035	66.39	28.4	92.31	91.27		
11.0021	66.30	28.4	92.65	92.78		
11.0106	66.86	28.4	92.43	90.04		

3.7. Determination of sample content

Take about 11.0 g of five batches of agar-15 powder samples, accurately weigh them, prepare the test solution according to the method discussed earlier, inject samples and analyze them respectively according to the chromatographic conditions discussed earlier, and calculate the content of isomatrine. The results are shown in **Table 3**. The experimental results show that the content of isomatrine in agar-15 powder is generally in the same range, and can be measured accurately and stably, which is of certain significance to drug quality control and quality supervision.

Table 3. Content determination results

Isomatrine content (μg/g)	RSD (%)
66.16	1.2
59.78	0.83
72.03	0.65
51.37	1.1
55.11	1.1

4. Discussion

4.1. Selection of mobile phase

Under the condition of isocratic elution, even if the proportion of organic phase in the mobile phase is compressed to 40%, the above six components cannot be well separated within a certain time. After trying to use gradient elution, isomatrine can be well separated from other components. Under this mobile phase, isomatrine has a higher separation degree, a higher number of theoretical plates and a more appropriate retention time.

4.2. Investigation of extraction time

Using methanol as extraction solvent, ultrasonic extraction was performed on two batches of samples for 15 min, 30 min, 60 min and 90 min, respectively. Through data analysis, it was found that the extraction amount of isomatrine increased with the extension of extraction time within 60 min. However, the extraction amount of 60 min isomatrine is roughly the same as that of 90 min isomatrine, so the final extraction time is 60 min.

Disclosure statement

The author declares no conflict of interest.

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Research on Nano-based Targeted Delivery Systems for Anti-tumor Drugs

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Abstract: Tumor metastasis and recurrence are significant factors leading to patient death, and chemotherapy drugs can cause severe damage to normal cells while killing tumor cells. Therefore, improving the efficacy of chemotherapy drugs and reducing their toxic side effects has become a research hotspot in recent years. Nanotechnology enables efficient targeting of drugs to tumor sites, enhancing the therapeutic effect of anti-tumor drugs. This article reviews the design of nano-based targeted delivery systems for anti-tumor drugs, including nucleic acid aptamers, protein polypeptides, surface modifications, and other nano-carriers and their construction strategies. It introduces various targeting binding systems based on different biomolecules (such as folic acid, hyaluronic acid, sugar chains, etc.) and ligands. This article summarizes the retention behavior of nano-carriers in the body and anticipates future trends in nano-based drug targeting delivery systems.

Keywords: Nanotechnology; Anti-tumor; Targeted drug delivery

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1. Introduction

Cancer is one of the major diseases threatening human life and health today. According to statistics, in 2022, there were approximately 4.82 million new cases of malignant tumors in China, with lung cancer ranking first, and esophageal cancer and colon cancer also accounting for a considerable proportion ^[1]. Currently, cancer treatment primarily includes surgery, chemotherapy, radiotherapy, and targeted drug therapy. Chemotherapy, which involves using drugs to induce apoptosis or death in tumor cells, is the most widely used anti-cancer method. Commonly used anti-tumor drugs include anti-angiogenic drugs, cytotoxic drugs, and endocrine-disrupting drugs ^[2,3]. However, their therapeutic effects are often unsatisfactory, and they have significant toxic side effects. Therefore, developing novel targeted anti-tumor drug delivery systems is crucial. Traditional drug delivery methods find it difficult to deliver anti-tumor drugs to the tumor site due to physiological barriers (such as vascular endothelial cells and smooth muscle cells) between tumor tissue and normal cells, significantly reducing the therapeutic effect of chemotherapy drugs. Targeted drug delivery systems can precisely deliver drugs into tumor cells, improving drug efficacy and reducing damage to

normal tissues and organs. In recent years, targeted drug delivery systems based on monoclonal antibodies and nucleic acid aptamers have garnered significant attention due to their high specificity and efficiency. However, these two targeting systems rely on antigens or nucleic acid sequences for coordination recognition, making it impossible to achieve a one-to-one correspondence between the drug and specific targets. Therefore, designing functionalized nanomaterials for targeted drug delivery is particularly important.

Nanotechnology refers to a technical system consisting of artificial structural units and their assemblies with sizes ranging from 1 to 100 nm, applicable in drug delivery, diagnostic testing, and gene therapy. TiO₂N is widely used in biomedicine due to its stable chemical properties, ease of synthesis, non-toxicity, good mechanical properties, and strong surface negativity. In recent years, researchers have used TiO₂ nanoparticles in cell imaging, immune regulation, DNA carriers^[4,5], and made some progress in anti-tumor research.

This article reviews the development status of nano-based targeted delivery systems for anti-tumor drugs from three aspects:

- (1) Introducing different types of nano-carriers and their construction strategies, including drug-loaded liposomes, polymer micelles, DNA self-assembled nanospheres, and nucleic acid aptamers;
- (2) Presenting targeting binding systems based on biomolecules and ligands, such as folic acid molecules, hyaluronic acid molecules, and sugar chain molecules;
- (3) Summarizing the retention behavior and metabolic kinetics of nano-carriers in the body to provide references for subsequent related research.

2. Nanocarriers and their construction strategies

Nanocarriers refer to nanoparticles with a size below 10 nm. They primarily bind to cell membranes or intracellular receptors through various means such as electrostatic interactions, physical adsorption, Van der Waals forces, and hydrogen bonding, thereby achieving drug delivery and release^[6]. Based on this, we refer to nanocarriers with targeting capabilities as drug-targeted delivery systems.

2.1. Aptamers

Nanoparticles are considered one of the most promising targeted delivery vehicles because they can specifically recognize specific sequences in proteins and elicit immune responses. As shown in **Figure 1**, a biological target can consist of one or multiple nucleic acid base pairs. These base pairs typically contain several complementary nucleotides (called complementary strands), and each base pair has a specific hydrophobic region. This conformational difference allows the two complementary strands to align in parallel but opposite directions, forming a “double-stranded lock” structure that is unstable and prone to breakage. Therefore, when aptamers specifically interact with target proteins, they can release a large number of amino acid residues, triggering protease cleavage or shearing reactions that lead to target protein inactivation^[7].

Based on this principle, researchers have developed various methods to design aptamers with high affinity and selectivity, including:

- (1) Artificial screening using known protein sequences^[8];
- (2) Synthesizing peptide chains containing multiple amino acids through protein engineering techniques^[9];
- (3) Screening nucleic acid fragments with high affinity for target proteins through phage display technology^[10];
- (4) Utilizing computer-aided molecular simulation techniques to predict aptamers with high affinity^[11].

In recent years, with the rapid development of sequencing technology, it has become possible to determine the complete genome information of any given species through genetic sequencing, thereby accelerating the research process of nano-targeted drugs. Currently, a large number of different types of aptamers have been reported, such as aptamers derived from human immunoglobulin G, neutrophil elastase, hemoglobin, etc. [12] Among them, some aptamers have been proven to effectively mediate tumor chemotherapy, gene therapy, and gene editing, making them one of the hot research directions in the current field of nanotechnology.

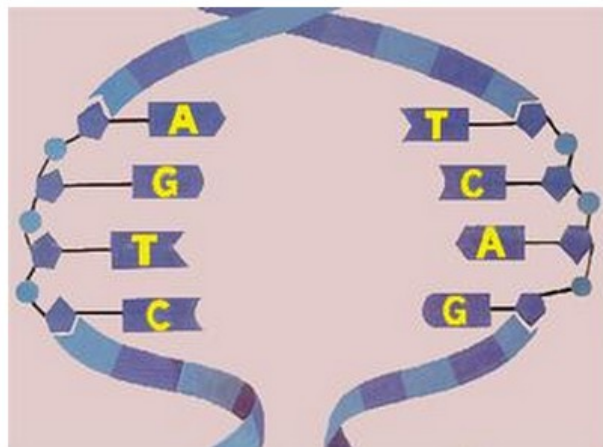


Figure 1. Nucleic acid base pairing.

2.2. Protein polypeptides

The surface of nanoparticles can achieve encapsulation of drug molecules through physical adsorption or chemical modification. Proteins themselves have good biocompatibility and biodegradability, so they are often used as drug carriers for drug delivery. For example, based on the reversible degradation characteristics of the tumor microenvironment, polyethylene glycol (PEG) can be grafted onto the cell membrane of tumor-associated fibroblasts and then encapsulated with chemotherapy drugs such as doxorubicin [13]. Some researchers have also designed and synthesized a new type of magnetic polymer microspheres using the electrostatic interaction between positively charged butylamine ions (NH_4^+) and negatively charged benzothiazole anions (BzS) to carry the anticancer drug paclitaxel [14]. These examples illustrate that using protein polypeptides to construct nanocarriers can not only reduce the residence time of drugs in the bloodstream but also significantly improve the retention efficiency of drugs at tumor sites.

2.3. Surface modification

To increase the stability of nanocarriers in organisms, surface modification is necessary. Common surface modification methods include lipidization, chemical modification, etc. For instance, lipidization can protect nanoparticles from damage by the body's enzyme system while reducing the risk of systemic exposure [15]. Additionally, by adding various functional molecules, the physicochemical properties of nanoparticles can be further improved, enhancing their stability in organisms and targeting effects [16]. Moreover, surface modification of nanocarriers helps address the issue of nanoparticles easily disintegrating under acidic conditions, ensuring long-term drug retention in tumor tissue.

3. Construction of target binding system

Dai *et al.* reported a nanosystem composed of folic acid and polymer carriers. This system covalently binds folic acid to polyethylene glycol (PEG) or hyaluronic acid with hydrophobic groups and attaches them to the surface of copper nanoparticles [17]. In the tumor microenvironment, tumor-associated fibroblasts abundantly express the folate receptor FFR1, enabling them to specifically recognize and ingest nanoparticles modified with folic acid, thereby facilitating drug release.

Yang proposed a carbohydrate-based targeting strategy, which utilizes the sugar chains in the tumor microenvironment for targeted binding [18]. Firstly, degradable carbohydrates such as glucose, galactose, and fucose are synthesized. These are then combined with cationic liposomes containing carboxyl or hydroxyl groups to prepare a targeted drug delivery system. These cationic liposomes can be modified using various methods to adapt to different types of cell surface receptors, such as membrane proteins or ion channels. Subsequently, cationic liposomes with different ligands are coated onto specific nanocarriers, ultimately obtaining nanocarriers with tumor-targeting properties.

Wei designed an amino-based lipophilic drug delivery system. This involves grafting a hydrophobic compound containing an amino group (such as tetrafluoroboric acid) onto polyethyleneimine. It is then introduced into polyethylene glycol through electrostatic interactions. Finally, mannose side chains are grafted onto the polyethylene glycol-tetrafluoroboric acid block copolymer [19]. Since the amino groups on the mannose side chains can specifically bind to mannose receptors on the surface of tumor cells, the mannose side chains can serve as a “molecular switch.” When ingested by tumor-infiltrating macrophages, the nanodrug carrier detaches from the polyethylene glycol backbone, releasing the previously enclosed drug and achieving efficient tumor treatment.

Additionally, researchers have designed a nucleic acid aptamer-based targeted binding system to enhance the specificity of drug targeting [20]. Nucleic acid aptamers select corresponding targets based on the affinity between their base sequences and tumor-related antigens, thus avoiding unnecessary toxic side effects caused by nonspecific binding. Simultaneously, nucleic acid aptamers can bind to a higher density of nucleic acid sequences, improving the accuracy of targeting tumor sites. Currently, several DNA-based nucleic acid aptamers have been successfully applied in clinical trials, such as Aptamer 437, which has shown significant therapeutic effects in the treatment of colorectal cancer.

4. Retention behavior of nanocarriers in the body

4.1. Passive targeting retention in tumor tissues

The retention behavior of nanocarriers in the body is primarily based on some special physiological and pathological characteristics of tumor tissues, among which the most typical is the Enhanced Permeability and Retention (EPR) effect. Due to rapid proliferation, tumor tissues are rich in neovascularization, have larger gaps between vascular endothelial cells, and lack an effective lymphatic drainage system. Nanocarriers with particle sizes in the range of 10–200 nm can penetrate tumor tissues through these enlarged vascular endothelial gaps and remain in the tumor site for a long time. For example, polylactic-co-glycolic acid (PLGA) nanoparticles, as commonly used nanocarriers, can passively accumulate in tumor tissues through the EPR effect when their particle size is within the appropriate range [21]. Studies have shown that doxorubicin loaded into PLGA nanoparticles has a significantly higher drug concentration in tumor tissues after intravenous injection compared to free drugs, and can maintain a higher concentration at the tumor site for a longer time, effectively improving the killing effect of the drug on tumor cells.

4.2. Active targeting retention

To further improve the retention efficiency of nanocarriers in tumor tissues, surface modification of nanocarriers can be performed to carry targeting ligands that can bind to specific receptors or antigens on the surface of tumor cells, achieving active targeting retention. Common targeting ligands include folate, transferrin, monoclonal antibodies, etc. Folate receptors are highly expressed on the surface of various tumor cells, and folate-modified nanocarriers can be efficiently taken up by tumor cells through specific binding to folate receptors [22]. For example, folate-modified liposomes encapsulating paclitaxel have significantly increased uptake in tumor tissues and prolonged retention time in tumor cells compared to unmodified liposomes, enhancing the inhibitory effect on tumor cells. Transferrin receptors are also overexpressed on the surface of many tumor cells, and transferrin-modified nanocarriers can also achieve active targeting and retention of tumor cells through receptor-mediated endocytosis [23]. Monoclonal antibodies have high specificity and can accurately recognize specific antigens on the surface of tumor cells. Connecting them to the surface of nanocarriers can significantly improve the targeting and retention time of nanocarriers at the tumor site. For example, trastuzumab-modified nanoparticles have good targeting and retention effects on HER2-positive breast cancer cells [24].

5. Conclusion

In the field of anti-tumor drug delivery, nanomaterials have attracted significant attention due to their unique physicochemical properties. However, designing nanocarriers with high targeting specificity, high drug loading capacity, and good biocompatibility remains a considerable challenge. Currently reported targeted carriers are mostly homogeneous systems that form stable encapsulated structures by wrapping ligands within the carrier after specific binding. As a result, these carriers often require dissolution with the assistance of organic solvents or other polymeric solvents. Nevertheless, organic solvents may cause severe adverse reactions in patients, while polymeric solvents may not achieve the desired targeting effect. Therefore, it is imperative to develop novel nanocarriers capable of achieving targeted delivery. This article primarily focuses on constructing drug targeting systems by exploiting interactions between different types of biomolecules and various target molecules. These molecules include folate receptors, hyaluronic acid receptors, sugar chains, proteins, and more. Among them, nucleic acid aptamer-based targeting systems are the most common. However, their development is limited by drawbacks such as susceptibility to degradation by host cells and the inability to penetrate the blood-brain barrier.

In future research, it is essential to delve deeper into the synergistic effects among different types of biomolecules and optimize their structures and properties for better application in drug targeting. Additionally, beyond drug targeting systems based on target molecules, there is potential to develop targeting peptides and drug delivery systems based on protein-ligand coupling strategies to further enhance the accumulation efficiency of drugs in the body.

Disclosure statement

The author declares no conflict of interest.

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