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Original Article

Exploring the mechanism of cytisine in treating respiratory depression following venomous snake bites based on network pharmacology and molecular docking

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Abstract: Objective: To investigate the antivenom mechanism of cytisine through network pharmacology and molecular docking (MD) techniques, with the intention of exploring its clinical applications. Methods: The cytisine target and the snakebite respiratory inhibition target were obtained using the Swiss Target Prediction platform and the Gene Cards database. The two target sets were overlapped to form a protein interaction network. Additionally, pathway enrichment analysis was conducted on cross targets, and the related pathways for the treatment of snake venom-induced respiratory failure were obtained. Verification of the MD between cytisine and its related targets was performed using the Autodock 1.5.7 software. The respiratory depression model of rats bitten by venomous snakes was established, and the expression of key target genes in the rat model was verified by western blot (WB). Result: A total of 16 targets of cytisine and 9 potential targets of cytisine in treating snake venom-induced respiratory depression were obtained. Core targets including CHRNA7, CHRNG, CHRNB1, CHRND, CHRNA1 and DRD2 were obtained. These targets are mainly enriched in neuroactive ligand-receptor interaction pathway and cholinergic synaptic pathway. The MD results demonstrated favorable docking activity of cytisine with its related targets. WB experiments showed that snake venom reduced the levels of CHRNA7 and CHRNG. Treatment with serum and cytisine could slow down this decline. Conclusion: Cytisine may synergistically target CHRNA7, CHRNG, CHRNB1, CHRND, CHRNA1, DRD2 and other proteins, modulating cholinergic and neuroactive pathways to alleviate neuromuscular block and protect acetylcholine receptors.

Keywords: Network pharmacology, molecular docking (MD), cytisine, respiratory depression, venomous snake bite

Introduction

Snakebite is a typical emergency in the tropics and subtropics, and snake activity peaks from March to December each year [1]. In China, the common snakes are mainly the Bungarus multicinctus and cobra [2]. These snakes primarily release neurotoxins that block synapses, resulting in symptoms such as muscle weakness, ptosis, ophthalmoplegia, speech and swallowing difficulties, and dyspnea in bitten patients. Severe poisoning can also lead to respiratory depression and even death [3, 4]. Clinically, the antivenom serum is often used to alleviate poisoning symptoms. However, it only neutralizes free toxins in the circulation but does not effectively treat organ damage or respiratory failure. Mechanical ventilation is an effective means to treat respiratory failure. Many clinical studies have also confirmed that mechanical ventilation has proven to be an effective treatment for respiratory failure in snakebite cases [5, 6]. However, the discomfort and risk of ventilator-associated pneumonia associated with mechanical ventilation, coupled with the unaffected state of consciousness in snakebite patients, necessitate the development of more effective adjuvant therapies. Cytisine, an alkaloid extracted from the seeds of the Leguminosae family, particularly Laburnum anagyroides, is a white powder with the molecular formula of $C_{11}H_{14}N_2O$ and a molecular weight of 190.24. It is soluble in acetone under weak alkali conditions and is extremely easy to oxidize, necessitating dark conditions during extraction. Intravenous or

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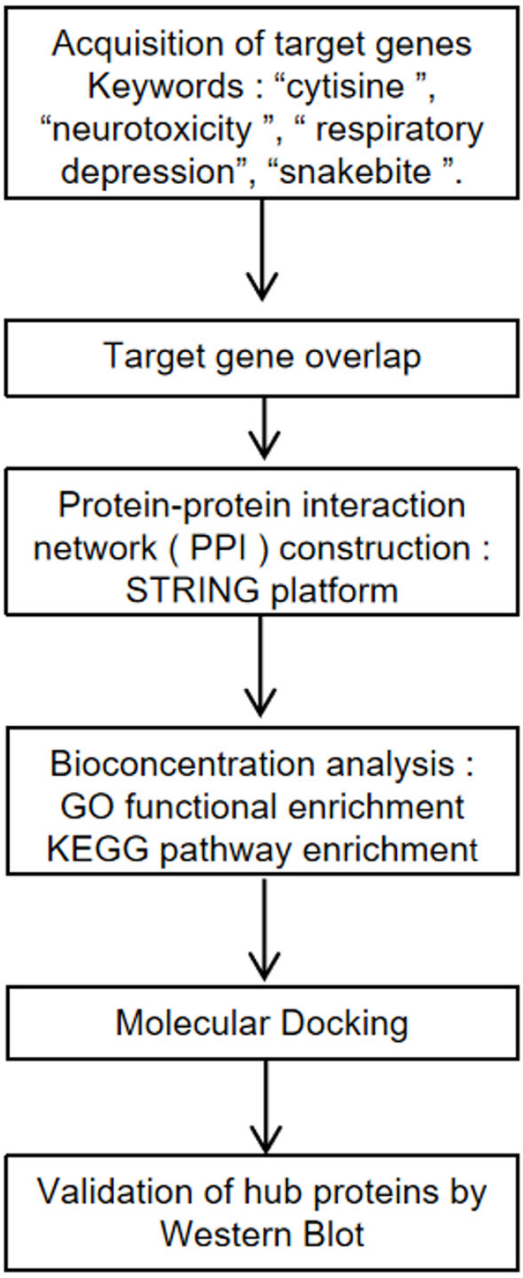

Figure 1. Research flow chart. PPI: Protein-Protein Interaction, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes.

intramuscular administration of a 0.15% cytisine solution in aqueous medium is commonly used in clinical settings to alleviate reflex apnea, shock, neonatal asphyxia, and facilitate smoking cessation by blocking nicotine-induced dopamine release [7, 8]. Based on the results of previous literature review, cytisine has the potential to treat respiratory depression, but whether it can improve respiratory depression after snakebite poisoning is not clear. Network pharmacology is a novel technology that integrates systems biology, biological network balance, proteomics, genomics, and other disciplines to explain the mechanism of drug action and the development of diseases from the perspective of “drug-disease-target-pathway”. This study explores the action pathway of cystine in treating snakebite induced respiratory depression through network pharmacology, aiming to uncover its mechanism via molecular docking verification and Western Blot, thereby highlighting its scientific relevance. The flow chart of this study is shown in **Figure 1**.

Materials and methods

Target gene identification of cytisine in treating snakebite-induced respiratory depression

To obtain the chemical properties and structural information of cytisine, we accessed the PubChem database and utilized the corresponding “Canonical SMILES” information to download all relevant target genes of Cytisine from the target prediction platform (SwissTargetPrediction).

While investigating the mechanisms of respiratory depression following venomous snake bites, we recognized that it is primarily caused by neurotoxin-induced obstruction of the respiratory system. To identify the relevant targets more precisely, we conducted searches in the GeneCards database using not only the keywords “neurotoxicity” and “respiratory depression” but also specifically included “snakebite”. Using the Venny 2.1 website, we performed an intersection analysis of these target information sets to determine the common targets.

In addition to collecting targets through platform tools, we also reviewed recently published literature to gather additional relevant targets mentioned therein. After clarifying the drug action and disease targets, we used the Venny 2.1 website once again to find the common targets of cytisine in the treatment of respiratory depression induced by snake venom.

Construction of protein-protein interaction networks (PPI)

The genes targeted by cytisine in the treatment of respiratory depression following a poisonous

snake bite were uploaded onto the STRING protein analysis platform, and the multi-protein analysis was chosen. The lowest binding score was selected as 0.7, and the PPI file was saved after removing the non-interacting targets. The PPI file was opened in Cytoscape 3.9.1 software, and the network topology parameters were calculated. The node size and color depth in the PPI network were set to change with the node degree size, and the line thickness between the connecting nodes was changed with the combined score size to determine the core target protein in the network.

Bioconcentration analysis

To clarify the mechanism of cytisine in treating respiratory depression after snakebite, it is necessary to perform biological enrichment analysis on the targets of drug treatment diseases, mainly involving GO functional enrichment and KEGG pathway enrichment. These analyses were carried out utilizing the David database, with an established criterion of $P < 0.05$ to determine significant enrichment relationships.

Molecular docking

In the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), the keyword 'cytisine' was used to retrieve its small molecular structure file. Additionally, the core protein structure file of respiratory depression after venomous snake bite, treated by malachite, was acquired from the uniport database and the PDB protein database. The protein file was preprocessed by Pymol software to remove excess ligands and water molecules. Hydrogenation. Autodock 1.5.7 software was used to complete the docking verification of small molecule cytisine and protein. Docking binding energy below 0 kcal/mol confirmed the presence of docking activity, while a value below -5 kcal/mol indicated a stronger docking effect [9].

Animal experiments

Additionally, 24 SPF grade Sprague Dawley (SD) rats with a male-to-female ratio of 1:1 were used as experimental animals. The rats were randomly assigned to four different experimental groups: blank control group, Bungarus multicinctus bite model group, model + serum treatment group, and model + serum + cytisine treatment group.

Establishment of Bungarus multicinctus venom poisoning model: Rats received Bungarus multicinctus venom solution by intramuscular injection. The specific dose was determined according to the median lethal dose (LD_{50}) of Bungarus multicinctus venom. In the experiment, the LD_{50} of Agkistrodon halys venom in rats was calculated to be 0.0094 mg/kg. According to this dose, different doses may be used in the experiment to establish the model, such as 3 times LD_{50} (0.0282 mg/kg) as the experimental dose to ensure the stability and repeatability of the model.

Intervention measures: Anti-Bungarus multicinctus venom serum: 1 hour after the establishment of the model, rats were injected with anti-Bungarus multicinctus venom serum through the tail vein. The serum dose was adjusted according to the experimental needs and the body weight of the rats. For example, 0.18 mL of serum per rat may be used. This dose was calculated based on the rat body weight (200 g) and serum concentration (10,000 units/mL).

Cytisine: The injection dose of genistein was calculated based on literature reports [10] and the body surface area of rats. For example, for 200 g rats, the dose of cytisine was calculated as 0.0092 mg (0.02 mg/kg × 2 kg × 0.23/0.2 kg = 0.046 mg/kg), and then the actual injection dose was adjusted according to this calculation result.

Western Blot (WB) assay: At the 4-hour time point, eyelid muscle tissue samples were collected from each group of rats. The samples were rapidly frozen in liquid nitrogen, ground into a fine powder using a pre-cooled grinding tool and transferred to a microtube containing a lysis buffer. The ratio of lysis buffer volume to tissue weight is typically 10:1. The microtubules were placed on ice for 30 minutes to complete protein lysis. They were then centrifuged at 12,000 rpm for 10 minutes to collect the supernatant as a protein sample. The BCA protein concentration assay kit was used according to the manufacturer's instructions. The protein samples were mixed with the BCA working solution and incubated at 37°C for 30 minutes. The protein concentration was calcu-

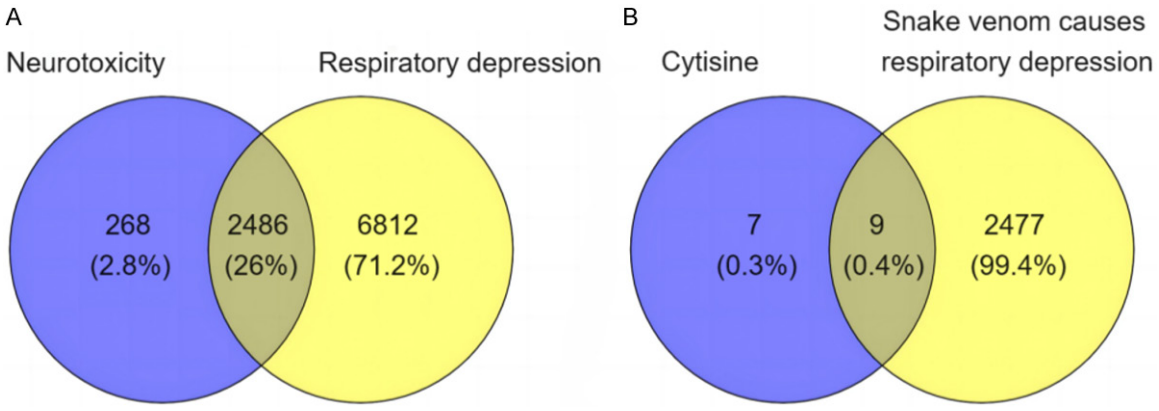

Figure 2. Venn diagram of intersection gene. (A) is the intersection of the neurotoxicity target and respiratory inhibition target; (B) is the intersection of cytisine and snake venom-induced respiratory depression.

lated according to the standard curve by measuring the absorbance at a wavelength of 562 nm using a microplate reader. To ensure complete denaturation of the protein, the protein sample was mixed with 4-fold loading buffer and heated in a water bath at 95°C for 15 minutes.

A 10% SDS-PAGE separation gel and a 4% stacked gel were prepared and combined. The separation gel was layered on top of the stacking gel, rinsed with distilled water, and then loaded with samples and protein standards. Electrophoresis buffer was added to the electrophoresis tank, ensuring the gel device was functioning properly before initiating the electrophoresis process. The initial voltage was set to 100 V. Once the dye enters the separation gel, the voltage was increased to 180 V until the dye reached the bottom of the gel. A transfer device was prepared, and the PVDF membrane was soaked in the transfer buffer. The gel and PVDF membrane were placed in the transfer device to ensure close contact. The transfer was conducted at a constant current of 200 mA at 4°C for approximately 120 minutes. Non-specific binding sites on the PVDF membrane were blocked using 5% skimmed milk powder or blocking solution and incubated for 1 hour. The PVDF membranes were incubated overnight at 4°C with diluted primary antibodies (CHRNA7 and CHRNG). After removing the unbound primary antibody, the membrane was incubated with the secondary antibody at room temperature for 2 hours. The membrane was then washed again and prepared for development. Subsequently, the PVDF membrane was immersed in an ECL developer for 1 minute then imaged on X-ray film, capturing the signal with a chemiluminescence imaging system. Quantitative analysis of the protein bands was performed to detect the expression level of the key proteins through image analysis.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. The database was established using all of the original data. The normality of the measurement data was tested using the Kolmogorov-Smirnov method. Measurement data with a normal distribution were presented as mean ± standard deviation and analyzed using one-way ANOVA. The measurement data with non-normal distribution were expressed as median (quartile) [M(P_{25}, P_{75})]. Non-parametric tests were used for comparison between groups. A significance level of $P < 0.05$ was considered statistically significant.

Results

Target genes of cytisine in the treatment of respiratory depression after snakebite

Using the SwissTargetPrediction platform and other literature reports, a total of 16 corresponding targets of cytisine were obtained. Using the GeneCards database and other literature reports, 2754 targets of “neurotoxin” and 9298 targets of “respiratory depression” were obtained. The intersection of the two yielded 2486 targets of respiratory depression caused by snake venom (**Figure 2A**). Subsequently, these targets were intersected with the 16 corresponding targets of cytisine and yielded nine targets of cytisine, including CHRNB1, CHRNA1, CHRNG, CHRND, CHRNA7, DRD2, PARP1, PNP

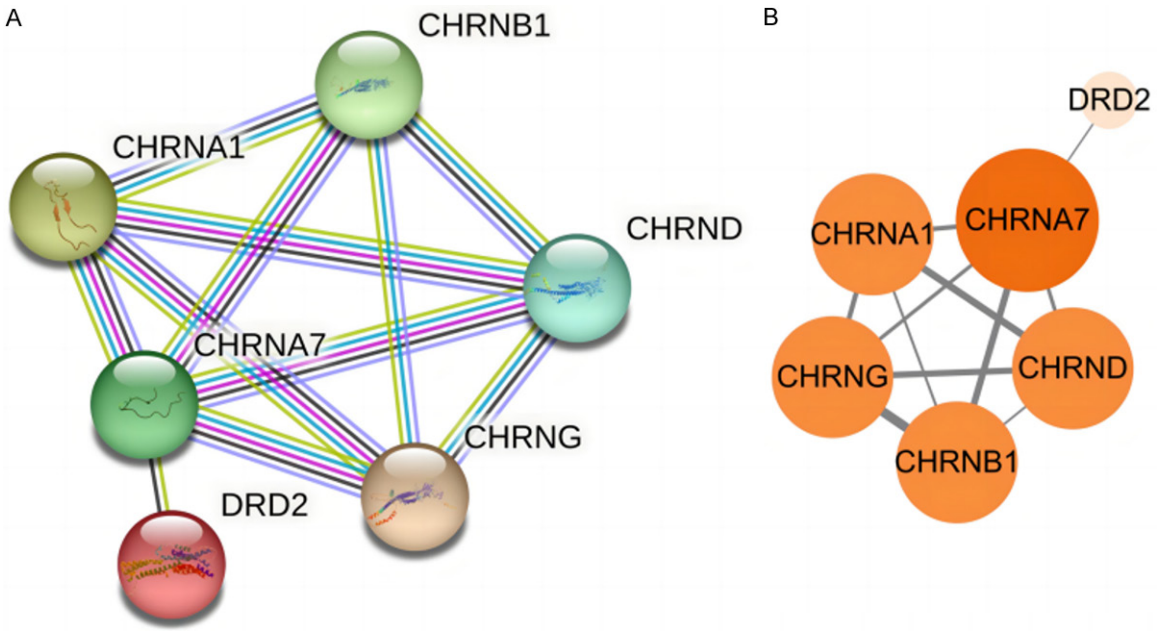

Figure 3. PPI network of cytisine in treating respiratory depression caused by snake venom. A: Interaction among the 6 proteins; B: Node size and color depth changed continuously with the degree value, and the interaction line thickness changed continuously with the combined score.

Table 1. Topological parameter table of 6 targets

Gene	Degree	MCC	Betweenness	Closeness
CHRNA7	5	25	8	5
CHRNG	4	24	0	4.5
CHRNB1	4	24	0	4.5
CHRND	4	24	0	4.5
CHRNA1	4	24	0	4.5
DRD2	1	1	0	3

and KISS1R, in the treatment of respiratory depression after snake bite (**Figure 2B**).

Construction of PPI network

The STRING protein analysis platform was used to establish a PPI network (**Figure 3A**) for the nine cytisine targets in treating respiratory depression caused by snake venom. After hiding the non-interacting proteins, only six targets (CHRNB1, CHRNA1, CHRNG, CHRND, CHRNA7, and DRD2) were displayed in the network diagram. The network diagram was calculated and analyzed by Cytoscape 3.9.1 software. The results showed that there were six nodes and 11 edges in the network diagram (**Figure 3B**). Moreover, it provided information on each node's degree, maximum cluster center value (MCC), betweenness value (Betweenness), and closeness value (Closeness). CHRNA7 was identified as the core target, as shown in **Table 1**.

Bioconcentration analysis

The GO enrichment analysis results indicated that there were 19 biological processes (BP), 10 cellular components (CC), and 10 molecular functions (MF). The biological processes concentrated on the excitatory postsynaptic potential in the neurological system process, membrane potential regulation, and synaptic transmission. Additionally, the choline pathway and the acetylcholine receptor signaling pathway were also found to be crucial (**Figure 4**). The primary cell components consisted of the postsynaptic membrane, the acetylcholine-gated channel complex, the synapse, the neuron projection, and the neuromuscular junction (**Figure 5**). The molecular function mainly involved acetylcholine-gated cation-selective channel activity, excitatory extracellular ligand-gated ion channel activity, acetylcholine binding, acetylcholine receptor activity, neurotransmitter receptor activity and so on (**Figure 6**). KEGG enrichment analysis showed that there were only three significantly enriched pathways, namely Neuroactive ligand-receptor interaction, Nicotine addiction and Cholinergic synapse (**Figure 7**), and the related targets were

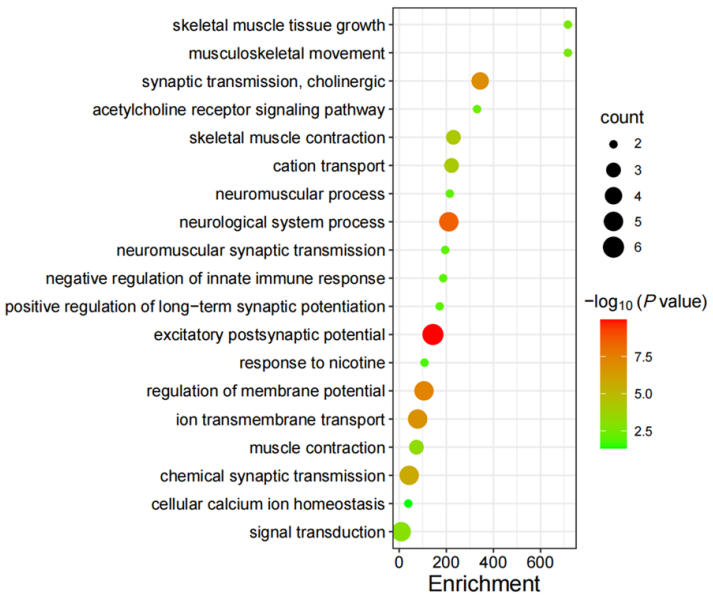

Figure 4. Bubble diagram of related target biological processes.

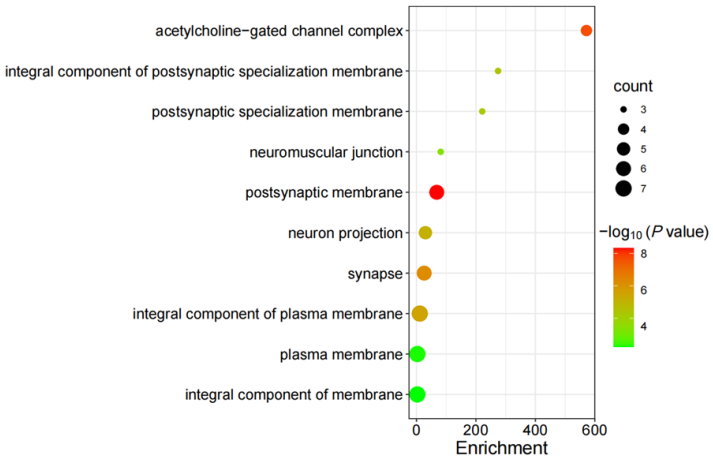

Figure 5. Bubble diagram of related target cellular component.

CHRNA1, CHRNB1, CHRND, CHRNG, CHRNA7, DRD2. The above analysis shows that the treatment of respiratory depression after venomous snake bite by cytisine primarily involves the neuroactive ligand-receptor interaction pathway, with acetylcholine transport and the regulation of synaptic membrane potential as key factors.

Molecular docking

In this study, the small molecule of cytisine, a drug component, was verified by MD with the six effective targets of CHRNB1, CHRNA1, CHRNG, CHRND, CHRNA7 and *DRD2*. The docking results showed that the minimum binding energy of the target protein and cytisine small molecule was between -4.5 kcal/mol and -7.0 kcal/mol, indicating a good binding between the target protein and cytisine (**Table 2**; **Figure 8**).

WB verification

Compared to the model group, the levels of CHRNA7 and CHRNG increased significantly in both the model + serum group ($P < 0.05$) and the model + serum + cytisine group ($P < 0.05$) (**Figure 9**).

Discussion

In this study, a protein-protein interaction (PPI) network was established to identify the targets of cytisine in the treatment of respiratory depression caused by snake venom. The network diagram revealed six targets: CHRNA7, CHRNG, CHRNB1, CHRND, CHRNA1, and DRD2, ranked by degree. Most of the above targets involve acetylcholine receptors, which is consistent with the mechanism by which snake venom causes respiratory failure. Neurotoxin is one of the major components of snake venom. Its main function is to block the neuromuscular junction and cause flaccid paralysis, eventually leading to peripheral respiratory failure, hypoxic encephalopathy, pulmonary infection and circulatory failure [11]. By acting on the presynaptic and postsynaptic sites of motor nerve endings, the neurotoxin mainly inhibits acetylcholine receptors on the motor endplate, so that acetylcholine, the neurotransmitter in the muscle, cannot exert its original depolarizing effect, resulting in striated muscle relaxation and respiratory depression [12]. The β-bungarotoxin in the venom of Bungarus multicinctus is a neurotoxin that exerts a presynaptic effect by inhibiting the release of neurotransmitters [13]. *CHRNA7* (Cholinergic Receptor Nicotinic Alpha 7 Subunit)

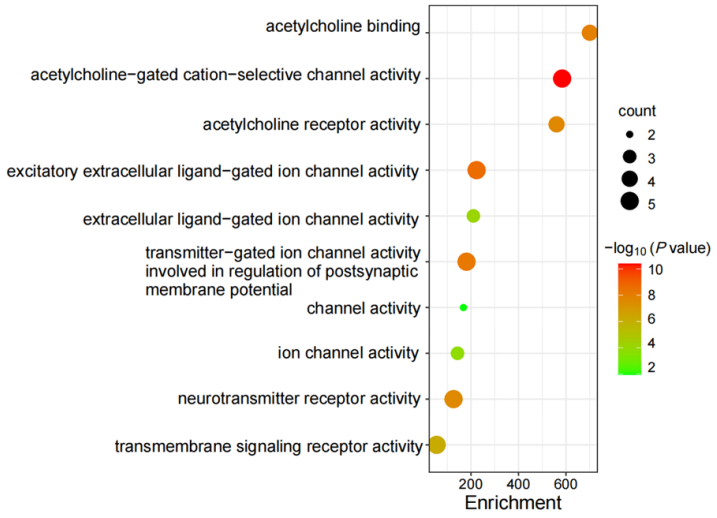

Figure 6. Bubble diagram of related target molecular functions.

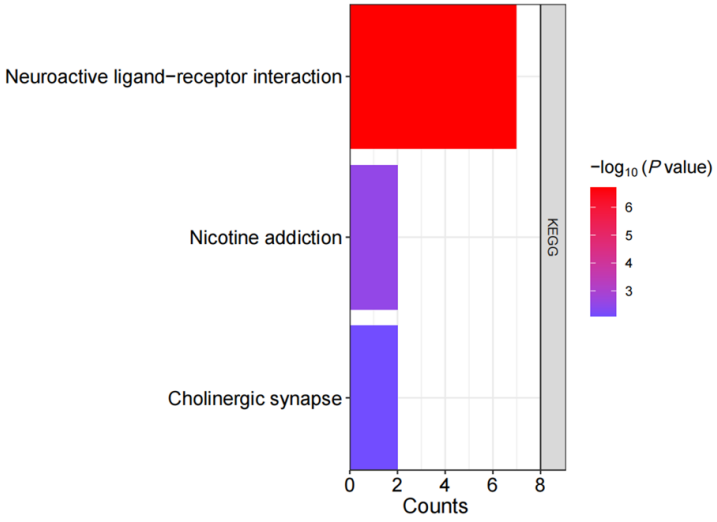

Figure 7. Related target Kyoto Encyclopedia of Genes and Genomes bars.

Table 2. Binding energy of cytisine with related proteins

Docking small molecule-large protein	Minimum binding energy (kcal/mol)
Cytisine-CHRNA7	-6.60
Cytisine-DRD2	-6.82
Cytisine-CHRNA1	-5.13
Cytisine-CHRNB1	-5.08
Cytisine-CHRND	-4.97
Cytisine-CHRNG	-4.96

is a neurodevelopmental protein involved in nerve differentiation and growth. It has been pointed out that *CHRNA7* is susceptible to α-bungarotoxin [14]. In recent years, this gene has been widely reported to be related to schizophrenia and Alzheimer's disease. Neri M et al. [15], reported that acetylcholine receptor dysfunction is often observed in patients with cognitive and mobility impairments, highlighting the critical role of CHRNA7 in neuronal physiology, development, and functional changes. Therefore, it is plausible that there is a specific correlation between snake venom-induced neurological impairments and CHRNA7. Similarly, CHRNA1 (Cholinergic Receptor Nicotinic Alpha 1 Subunit) is also involved in neurodevelopment, neuroplasticity, and the development of mental disorders (including bipolar disorder). Recently, Liao Z et al. [16] reported that CHRNA1 was involved in the induction of osteoporosis in the elderly, suggesting that osteoporosis in the elderly was a neurodegenerative muscle disease, and the up-regulation of CHRNA1 expression reduced muscle action potential and muscle contraction ability. The Irumudomon O team [17] also pointed out that CHRNA1 was closely related to the structure and function of the neuromuscular junction (NMJ). This gene mutation may lead to congenital myasthenia syndrome in children. Similarly, CHRNB1 (Cholinergic Receptor Nicotinic Beta 1 Subunit) [18], CHRNG (Cholinergic Receptor Nicotinic Gamma Subunit) [19], and CHRND (Cholinergic Receptor Nicotinic Delta Subunit) have also been reported to be involved in the occurrence and development of myasthenia symptoms. It is evident that acute paralysis following snakebite is a neuromuscular emergency, with more than 20% of the patients experiencing muscle weakness, manifested as acute respiratory depression in severe cases [20]. This study underscores that these genes are closely related to the nerve paralysis caused by

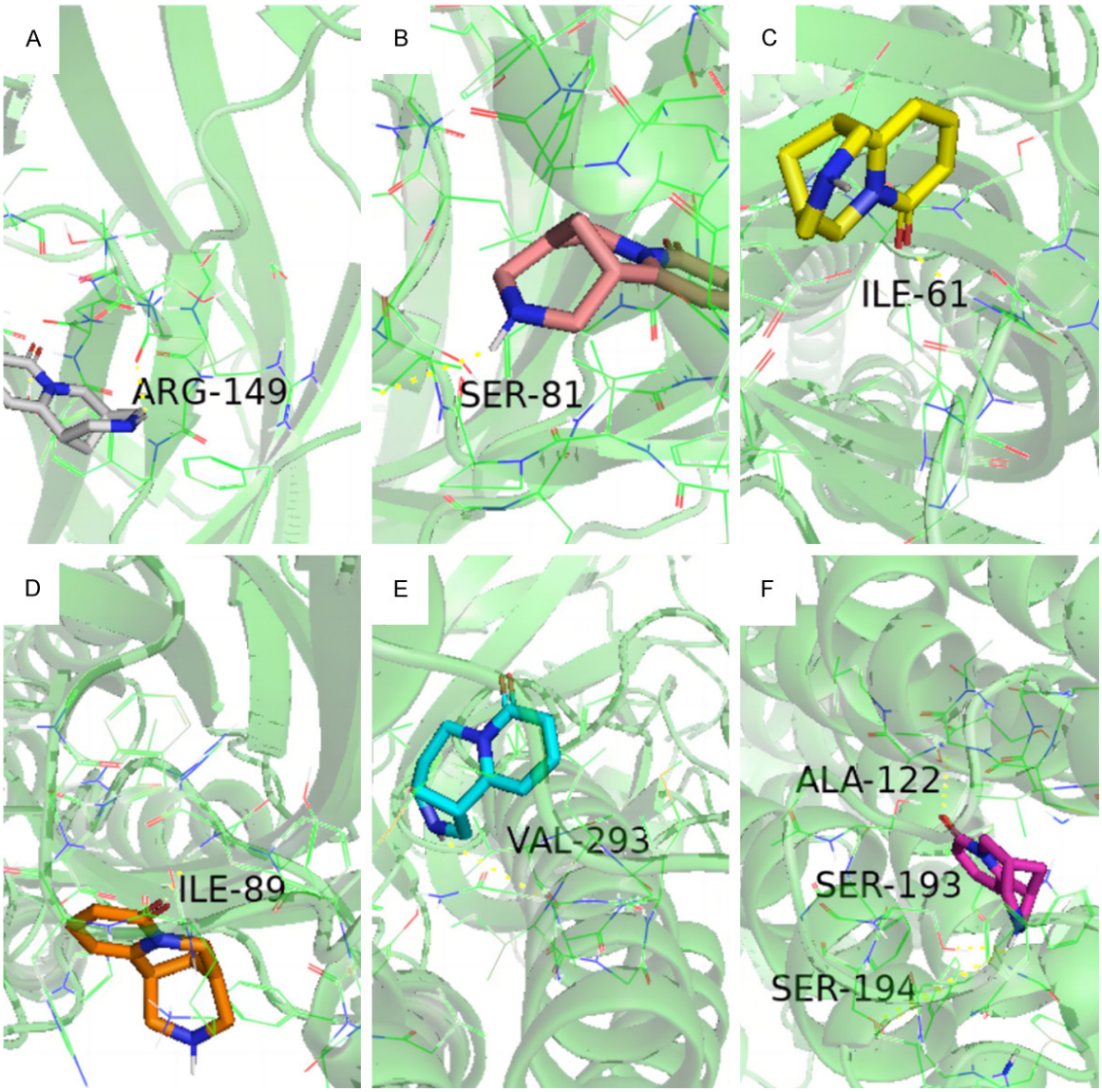

Figure 8. Docking diagram of cytisine and related proteins (the docking amino acid residues are shown in the diagram). A: Cytisine-CHRNA1; B: Cytisine-CHRNA7; C: Cytisine-CHRNB1; D: Cytisine-CHRND; E: Cytisine-CHRNG; F: Cytisine-DRD2.

snake venom induced respiratory depression. DRD2 refer to dopamine receptor D2. Studies have found that snake venom affects nicotinic acetylcholine receptors (NAChRs) and damages dopaminergic neurons. The joint action may cause muscle tremors or paralysis and endanger the central respiratory system [21].

The biological enrichment analysis of critical targets revealed that cytisine treatment for respiratory depression following snakebite mainly involves postsynaptic membranes, synapses, acetylcholine-gated channel complexes, neuromuscular junctions, neuron projections, and other components that mediate excitatory postsynaptic potentials, neurological system processes, regulation of membrane potentials, and synaptic transmission. Furthermore, biological processes such as cholinergic and acetylcholine receptor signaling pathways. KEGG pathway analysis showed that the effective targets were mainly enriched in the neuroactive ligand-receptor interaction pathway and cholinergic synapse pathway. In general, neuromuscular conduction undergoes several processes, including: (1) Voltage-gated calcium (Ca^{2+})

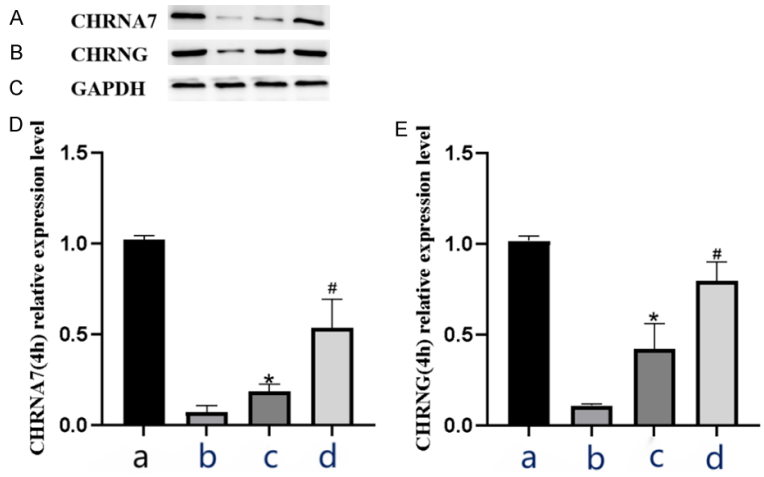

Figure 9. Expression level of acetylcholine receptor signaling pathway-related proteins detected by WB in rats (4 h). Note: A: CHRNA7 protein band; B: CHRNG protein band; C: Internal reference protein band; D: Comparison of CHRNA7 protein expression in 4 groups of rats in 4 h; E: Comparison of CHRNG protein expression in 4 groups of rats in 4 h; *P < 0.05 versus model group, #P < 0.05 versus model + serum group, a = Control group, b = Model group, c = Model + serum group, d = Model + serum + cytisine group.

channel opening on nerve endings; (2) The creation of a fusion complex consisting of SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) and the facilitation of synaptic vesicle fusion and ACh (acetylcholine) release; (3) ACh binds to nicotinic acetylcholine receptors (nAChRs) on the postsynaptic membrane [22], leading to the influx of sodium (Na^+) and calcium (Ca^{2+}), accompanied by potassium (K^+) outflow through K^+ channels. In addition, there are also nAChRs on the nerve endings, that is, presynaptic neuronal nAChRs [23]. After activation, it can be transported from the 'storage pool' to the 'release pool' by mobilizing ACh to promote the release of ACh finally. The released ACh quickly diffuses in the synaptic cleft and is eventually degraded by acetylcholinesterase (AChE). Neuromuscular block caused by snake venom occurs at these critical sites. Studies have found that the main target of cytisine is neuronal nicotinic acetylcholine receptors (NAChRs). The acetylcholine receptor signaling pathway interacts with various nAChR subtypes in different central and peripheral nervous system regions [8]. The neuroactive ligand-receptor interaction pathway has been reported to be involved in the pathogenesis of neurodegenerative diseases. Kong Y et al. [24] suggested that certain miRNAs are highly likely to have an impact on the neuroactive ligand-receptor interaction pathway. Among them, miR-137 is anticipated to regulate the majority of identified targets within the pathway, including DRD2. Neurotoxic reactions caused by neurodegenerative diseases can be seen in the down-regulation of miR-137 and its target genes. By regulating the expression of related miRNAs, the death of dopaminergic neurons can be reduced to maintain the stability of nervous system function. Cholinergic synaptic pathways have been extensively studied in respiratory diseases, demonstrating that the regulation of the cholinergic system plays a crucial role in bronchial contraction among asthma patients. In addition, the interaction between ACh and the trigeminal pararespiratory group (PTRG) has been shown to affect respiratory activity, emphasizing the importance of the cholinergic system in respiratory function [25, 26]. Overall, cytisine may aid the preservation of the acetylcholine signal function by regulating the pathways of acetylcholine receptor signaling, neuroactive ligand-receptor interaction, and cholinergic synaptic. Furthermore, it can also reduce the damage caused by neurotoxins produced by snake venom to related neurons, and potentially treat respiratory depression caused by snake venom.

In our study, we selected CHRNA7 and CHRNG proteins for WB verification, which was based on the significant difference in their binding energy with cytisine in the molecular docking results: CHRNA7 exhibited the lowest binding energy, while CHRNG exhibited the highest binding energy. We hypothesized that these extreme values of binding energy may reflect the importance and sensitivity of these proteins in biologically interacting with cytisine. The results showed that the snake venom could reduce the levels of CHRNA7 and CHRNG in rats, and the therapeutic effect of serum and cytisine could slow down this decrease. Therefore, the expression levels of CHRNA7 and CHRNG were closely related to the potential therapeutic mechanism of cytisine. The

CHRNA7-encoded nicotinic acetylcholine receptor α7 subunit (nAChRα7) plays a crucial role in the nervous system, specifically in regulating neurotransmission and neuroprotection. Snake venom-induced respiratory depression may interfere with the normal function of the neuromuscular junction, resulting in the inhibition of acetylcholine release and causing neuromuscular block due to neurotoxins [27]. Cytisine is an agonist for nicotine acetylcholine receptor, specifically activating nAChRα7 and enhancing acetylcholine signal transduction. This helps to restore neuromuscular transmission and alleviate neuromuscular block caused by snake venom, specifically respiratory depression. Additionally, nAChRα7 is associated with neuroprotective and anti-inflammatory effects [28]. Recent studies have revealed the key role of α7nAChR in the cholinergic anti-inflammatory pathway (CAP), particularly in reducing the severity of acute respiratory distress syndrome (ARDS) [29]. The above study shows that mesenchymal stem cells (MSCs) can reduce pulmonary inflammation and improve respiratory symptoms in ARDS patients by activating α7nAChR. It is speculated that cytisine may exert its therapeutic effect through a similar CAP mechanism, as it is known to activate nAChRα7 [30].

The nicotinic acetylcholine receptor γ subunit (nAChRγ), encoded by CHRNG, is a crucial element of various nAChR complexes. Our investigation into the potential mechanism of cytisine in treating snake venom-induced respiratory depression revealed that the neuromuscular junction (NMJ) dysfunction in congenital myasthenia syndrome (CMS) is similar to respiratory disorders. In a review by Ohno et al. [31], they provided a detailed description of the crucial role played by the nAChRγ gene in neuromuscular transmission: the activation of nAChRγ enhances acetylcholine signalling and reduces neuromuscular block, thereby improving damaged nerves. Cytisine may enhance the function of nAChRγ. In addition, Ohno et al. also highlighted the effectiveness of cholinesterase inhibitors in most CMS patients, but not in CMS caused by certain gene mutations. This suggests that we have to consider the specific genetic background of patients when considering cytisine as a strategy for the treatment of respiratory depression caused by snake venom. Although WB experiments only verified some of the target proteins, we recognized that experimental verification of other hub genes (such as CHRNB1, CHRND, CHRNA1, DRD2) is equally important. Therefore, we plan to conduct further experimental verification of these genes in future studies to comprehensively evaluate the mechanism of action of cytisine.

Conclusion

In summary, this study applied network pharmacology methods and molecular docking techniques to theoretically explore the complex mechanism of cytisine in treating respiratory depression caused by snake venom. Cytisine exhibits a synergistic effect on disease-related target proteins, including *CHRNA7, CHRNG, CHRNB1, CHRND, CHRNA1*, and *DRD2*, leading to the regulation of pathways responsible for acetylcholine receptor signaling, neuroactive ligand-receptor interaction, and cholinergic synaptic activity. Consequently, cytisine consistently plays a role in alleviating neuromuscular conduction block and protecting acetylcholine receptor function.

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Disclosure of conflict of interest

None.

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