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Multiple biological functions and pharmacological effects of lycorine

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Lycorine is the major active component from the amaryllidaceae family plant *Lycoris radiate*, a represent traditional Chinese medicinal herb, and is one of the typical alkaloids with pyrrolophenanthridine nucleus core. Lycorine has drawn great interest in medicinal field due to its divergent chemical structures and multiple biological functions, as well as pharmacological effects on various diseases. Accumulated evidence shows that lycorine not only possesses strong pharmacological effects on many diseases, including anti-leukemia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and anti- malaria, but also exerts many other biological functions, such as inhibition of acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis, and control of circadian period length. Notably, lycorine exhibits its numerous pharmacological effects on various diseases with very low toxicity and mild side effects. The divergent chemical structures, multiple biological functions, and very low toxicity of lycorine imply that the agent is a potential drug candidate that warrants for further preclinical and clinic investigation.

traditional Chinese medicinal herbs, lycorine, anti-cancer, anti-virus, angiogenesis, neovascularization

1 Introduction

Traditional Chinese medicine (TCM) herbs have been used to treat various diseases in China for thousands of years, and got sound progress in treatment of leukemia in recent years. In particular, Realgar-Indigo naturalis formula, a represent ancient formula in China, has been verified to cure the patients suffered from acute promyelocytic leukemia (APL) with high efficacy and dramatic prolongation of five-year overall survival of the patients [1]. Arsenic trioxide, a key active component derived from an anti-cancer formula of TCM, has shown a promise in treatment of APL [2]. Furthermore, the combination of arsenic trioxide with all-*trans* retinoic acid (ATRA) effectively eliminates APL cells and dramatically improves the five-year overall survival rate of the leukemia patients. Accumulated evidence shows that TCM herbs are great treasure house for novel drug discovery [3]. Lycorine, derived from the TCM herb amaryllidaceae family plant *Lycoris radiate*, has drawn great interests in medicinal field due to its divergent chemical structures and strong pharmacological effects on various diseases.

Lycoris radiate, a famous plant in TCM herbs, is the squamous bulb of Amaryllidaceae family called “Shi Suan” in China. The herb has been widely used as ornamental and medicinal plant in China for thousands of years [4], and also utilized in folk medicine in many countries. It is recorded by a famous Compendium of Materia Medica in China that *Lycoris radiate* can be used to antidote the poison, relieve inflammation, alleviate pain, diminish phlegm, and act as a diuretic drug. In southern Africa, Amaryllidaceae family plants have been used to treat swelling of the body, urinary tract problems, and itchy rashes. Additionally, the plants have been utilized to stimulate milk production in women and cows [5].

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Amaryllidaceae family plants, in particular *Lycoris radiate,* are a widely reputable family for its exclusive group of alkaloids and have been studied for nearly 200 years [6]. Among these alkaloids, lycorine is the first active alkaloid of Amaryllidaceae family plants to be isolated from the plant *Narcissus pseudonarcissus* in 1877 [6], and has been extensively studied in its chemical molecule structures and biological functions (Figure 1). Lycorine possesses multiple pharmacological effects, including anti-tumor [7–12], anti-angiogenesis and tumor cell-mediated vasculogenic mimicry [11, 12], anti-virus [13–18], anti-bacteria [19–21], anti-inflammation [22–25], inhibition of acetylcholinesterase (AChE) [26–31], suppression of ascorbic acid biosynthesis [32–35], anti-malaria [36–39], control of period length [40], and exercise of choleretic effect [41]. More interestingly, lycorine has very low toxicity to normal cells and normal bodies at an effective dose. In this review, we will first introduce the resources, structure, and characters of lycorine; and then address multiple biological functions, mainly focusing on anti-leukemia, anti-tumor, anti-tumor neovascularization, and safety of lycorine; and finally discuss the perspectives of lycorine in translational medicine.

2 Resources, structure, and characters of lycorine

2.1 Resources of lycorine

Lycorine, a pyrrolophenanthridine alkaloid has been isolated and identified from alkaloids of medicinal amaryllidaceous plants, including *Lycoris radiate*, *Leucojum aestivum*, *Hymenocallis littoralis*, *Ammocharis coranica*, *Brunsvigia radulosa*, *Crinum macowanii*, and *Leucojum aestivum* [42–46]. Georgieva *et al*. [47] isolated and analyzed the major alkaloids from wild populations of amaryllidaceous plant *Leucojum aestivum*, and detected 19 alkaloids by the gas chromatography-mass spectroscopy (GC-MS) method . While the alkaloid fractions of *Leucojum aestivum* bulbs were rich in galanthamine type of compounds, lycorine and derived alkaloids were found as dominant compounds in the herb [42–46]. In addition, lycorine is a major alkaloid in *Crinum macowanii* plant and distributes in various parts of the herb. The amounts of lycorine in the bulb, root, leaf, flowering stalks of the plant are 5.937, 4.286, 4.244, and 4.279 mg/100g dry weight, respectively [47, 48]. Thus, lycorine can be purified from the amaryllidaceous plants [47, 48].

Although lycorine is a major alkaloid in amaryllidaceous plants, it is difficult to get a large amount of the purified agent to meet the great demand. Ptak *et al*. established an *in vitro* biosynthesis system to produce lycorine, and obtained 20 mg lycorine/100g plant (0.02%) in the tissue cultures of *Leucojum aestivum* [49]. Kaya *et al*. [50] established a reversed-phase HPLC method to purify and determine the contents of lycorine in Amaryllidaceae plants. Combination of the biosynthesis and analysis methods makes it possible to obtain purified lycorine in large quantities.

Although the biosynthesis and purification of lycorine provide a way to get a large amount of the agent, the cost is high; thereby, chemical synthesis of lycorine comes to the stage. Lycorine can be obtained by asymmetric synthesis or total synthesis of 2-epi-lycorine using a chiral ligand-controlled asymmetric cascade conjugate addition reaction [51, 52]. This methodology enables the formation of two C–C bonds and three stereogenic centers in one pot and gives synthetically useful chiral cyclohexane derivatives to obtain a large amount of lycorine. The synthetic strategy is flexible and applicable for other natural and unnatural lycorine derivatives [51, 52].

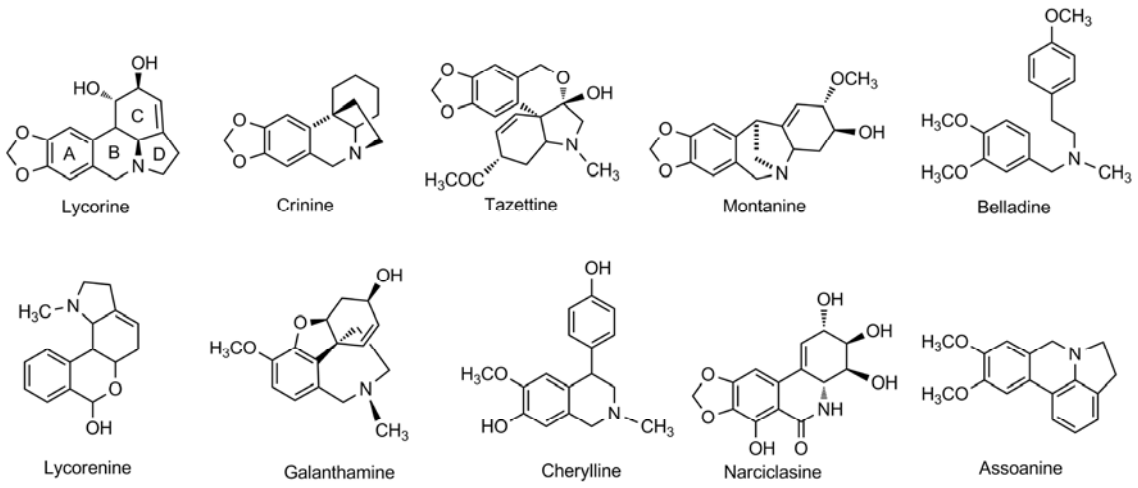

Figure 1 Divergent chemical structures of amaryllidaceae alkaloids.

2.2 Structure and characters of lycorine

All Amaryllidaceae alkaloids have a unique chemical structure, of which fundamental molecular ring system derives from L-phenylalanine and L-tyrosine, characterized by a single nitrogen atom. Amaryllidaceae alkaloids have divergent chemical structures and are consisted of nine chief different types of chemicals, including lycorine, crinine, tazettine, montanine, belladine, lycorenine, galanthamine, cherylline, and narciclasine (Figure 1) [53]. The lycorine type alkaloids have a typical pyrrolophenanthridine nucleus structure. The full chemical name of lycorine is 2,4,5,7,12b, 12c-hexahydro-l*H*-(1,3)dioxolo(4,5-j)-pyrrolo(3,2,1-de)phenanthridine-1-diol, and its molecular formula is $C_{16}H_{17}NO_4$ with the relative molecular mass of 287.31. Lycorine is a colorless prisms crystal with the melting point of about 260–262 °C, and it is very stable and can be preserved at room temperature for about three years [53]. Lycorine is immiscible to the wastewater and insoluble in ether and alcohol. The hydrochloride form of lycorine is a needle-shaped crystal with the melting point of about 217 °C.

3 Multiple biological functions and pharmacological effects of lycorine

Accumulated evidence shows that lycorine has multiple biological activities and pharmacological effects, including anti-leukemia, anti-tumor, anti-angiogenesis, anti-virus, anti-bacteria, anti-inflammation, and anti-malaria, inhibition of acetylcholinesterase and topoisomerase, suppression of ascorbic acid biosynthesis, and control of circadian period length (Figures 2 and 3), which will be reviewed in this section.

3.1 Anti-tumor effect of lycorine

Since Jimenez and colleagues found the anti-tumor cell proliferation of lycorine in 1976 [54], many researchers reported anti-leukemia and anti-cancer effects of lycorine. Liu *et al.* reported that lycorine inhibited cell proliferation and induced cell apoptosis in acute myeloid leukemia (AML) cell line HL-60 [55], monocyte leukemia cell line U937, and T-cell leukemia cell line Jurkat [56]. Lycorine also blocked multiple myeloma KM3 cell cycle [57], induced K562 cell-cycle arrest at the G_0/G_1 phase [58], and suppressed tumorigenesis and the growth of various tumor cells, including melanoma C8161 [11], ovarian cancer Hey1B [12], lung cancer A549, glioblatatoma U373, and esophageal cancer OE21 (Table 1) [9]. In addition, lycorine exerted antitumor activity against lung cancer LLC cells *in vivo*. When the LL2-xenographed mice were treated with lycorine at a dose of 10 mg/kg for 19 days, the tumor size was reduced for 80.5% as compared with the control [59]. Among the tumor cells treated with lycorine, HL-60 cells were one of the most sensitive tumor cell lines to the agent with ID_{50} of 1 μM [55]. *In vivo* studies, lycorine effectively inhibited tumor growth in several mouse xenograft models [12]. For examples, in HL-60 xenograft SCID mice, lycorine at doses of 5 or 10 mg/kg/day effectively inhibited occurrence of leukemia [60]. In metastatic ovarian cancer Hey1B xenograft nude mice, lycorine at dosage of 15 mg/kg

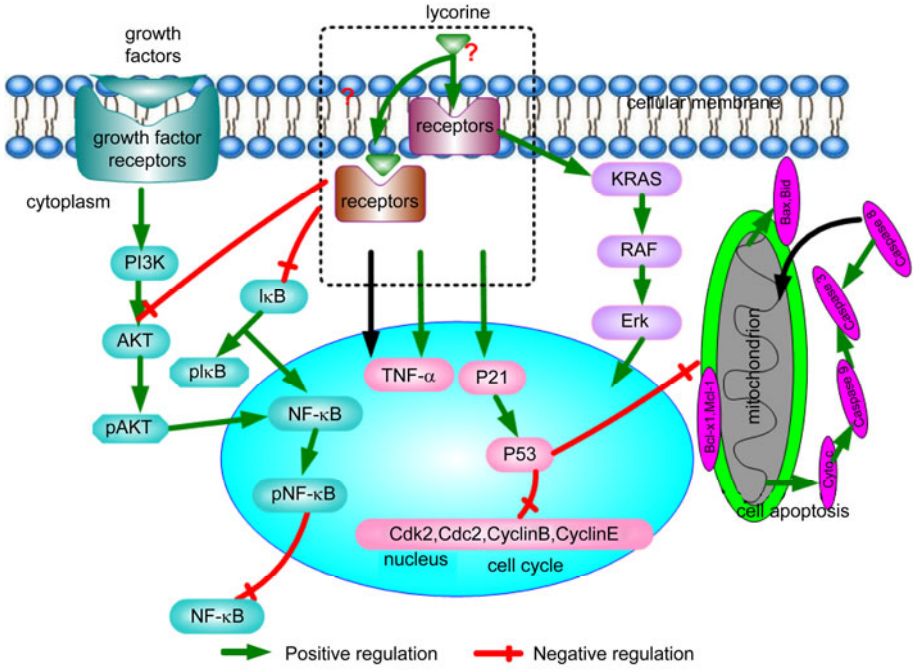

Figure 2 The mechanisms of lycorine exerted anti-cancer and anti-tumor neovascularization.

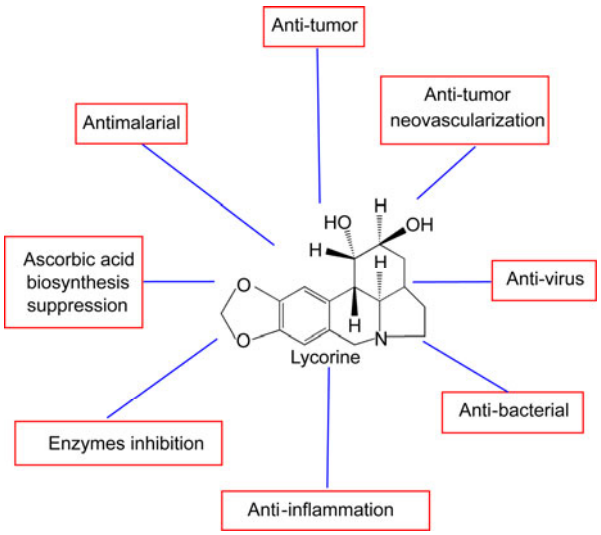

Figure 3 Multiple pharmacological effects of lycorine.

Table 1 IC50 values of lycorine on tumor cell lines

Tumor type	*Cell line*	IC50 (μM)	*References*
Leukemia	HL-60	1	[12, 17]
	K562	1.25	[60]
	U937	2.42	[17]
	CEM	1.6 ± 0.0	[11]
Ovarian cancer	Hey1B	1.2	[10]
Multiple myeloma	KM3	1.25	[59]
Breast adenocarcinoma	MCF-7	13.0 ± 2.9	[11]
Colon adenocarcinoma	HT-29	3.2	[12]
Hepatoma cell	HepG2	3.7	[12]
Melanoma	G-361	5.0 ± 0.3	[11]
	C8161	1.2	[10]
	SKMEL-28	8.4 ± 0.2	[8, 13]
	B16F10	6.3 ± 0.2	[8, 13]
Cervical adenocarcinoma	Hela	10.6 ± 0.9	[11]
Lung cancer	A549	4.2 ±0.4	[8, 13]
	LLC	0.5	[61]
Glioblastoma	Hs683	6.9 ± 0.5	[8, 13]
	U373	7.6 ± 0.4	[8, 13]
Eophageal cancer	OE21	4.5 ± 0.7	[8, 13]

suppressed 60% tumor volume as compared with the control [12]. In B16F10 melanoma-bearing mice, lycorine at dose of 40 mg/kg three times a week significantly increased the survival rate of the mice [9].

The mechanisms of lycorine-mediated anti-tumor are unclear. Emerging evidence shows that the main mechanisms are related to inhibition of tumor cell proliferation and induction of tumor cell apoptosis (Figure 2). Lycorine inhibits HL-60 cell proliferation by arresting cell cycle at the G_2/M phase, down-regulation of induced myeloid leukemia cell differentiation protein Mcl-1, and up-regulation of tumor suppressive proteins p21 and TNF-α [55]. In addition, lycorine is able to cause the multiple myeloma KM3 cell cycle at the G_0/G_1 phase through down-regulation of both cyclin D1 and CDK4 [57], inhibition of HDAC activity, and up-regulation of expressing tumor depressors p53 and p21 [58]. More recently, our own study uncovered that lycorine effectively inhibited mitotic proliferation of metastatic ovarian cancer Hey1B cells through enhancing expression of the cell cycle inhibitor p21 and suppressing expression of cyclin D3, resulting in cell cycle arrest at the G_2/M transition phase [12]. Lycorine also inhibited cell proliferation via down-regulation of expressing Cdc2, cyclin B, Cdk2, and cyclin E in leukemia cells [61]. Furthermore, lycorine is able to inhibit protein synthesis. For examples, the binding of $^{[3H]}$-narciclasine to yeast ribosomes can be inhibited by lycorine [31]. In a normal rat kidney epithelial cell (NRK) model, lycorine specially inhibits protein synthesis in the cultured oncogene K-Ras-transformed NRK cells through suppressing oncogenic K-Ras expression, but not obviously affects protein synthesis in the control NRK cells without K-Ras transformation, suggesting that lycorine has specific inhibitory effect on K-Ras-transformed cells and functions as K-Ras inhibitor [31]. It is well established that mutation and activation of K-Ras play a pivotal role in tumorigenesis of various malignant tumors, such as pancreatic cancer and lung cancer, hence, lycorine may be a potential candidate drug for targeted anti-cancer therapy in K-Ras dominant malignant tumors. These studies imply that lycorine is a potential anti-tumor drug.

Besides inhibition of tumor cell proliferation, lycorine also induces tumor cell apoptosis [8, 55, 62]. Mechanism studies show that lycoline promotes leukemia cell HL-60 apoptosis by increasing caspase-8, -9, -3 activities, decreasing expression of anti-apoptosis gene B-cell lymphoma 2 (Bcl-2), and elevating the level of pro-apoptotic Bcl-2 associated X protein (Bax) [55]. Furthermore, lycorine pronounces apoptosis accompanied by the down-regulation of Mcl-1 expression in myeloid blasts from the patients with acute myeloid leukemia [8]. Lycorine also fosters the truncation of BH3 interacting-domain death agonist (Bid), decreases I-κB phosphorylation, blocks NF-κB nuclear import, and promotes cytochrome C release from mitochondria (Figure 2) [61]. Moreover, Lycorine sensitizes CD40 ligand-protected chronic lymphocytic leukemia cells and enhances anti-cancer drug bezafibrate and medroxyprogesterone acetate-induced leukemia cell apoptosis [62].

3.2 Anti-tumor neovascularization of lycorine

In 1971, Folkman raised a theory of tumor angiogenesis and found that endothelial sprouting angiogenesis is a necessary condition for malignant tumor growth and metastasis [63]. Accordingly, anti-angiogenic drugs has been used in the clinics for anti-cancer therapy and gained a promise in treatment of several types of malignant tumors, such as co-

lon cancer and non-small cell lung cancer [64–66]. However, recent preclinical and clinical studies have shown that current anti-angiogenic drugs are a double-edged sword of anti-cancer therapy. The effect of anti-angiogenesis is shortly lived in majority of the cancer patients, and the long term benefit of anti-angiogenic therapy has not materialized in most of the patients. Unfortunately, the cancer patients develop drug resistance and some of them have cancer metastasis after anti-angiogenic therapy [67–72]. Thus, new anti-tumor angiogenic strategy and approach are highly desired [73, 74].

Increasing evidence shows that tumor may produce own vasculature through multiple mechanisms, including endothelial sprouting angiogenesis, bone marrow endothelial progenitor cells (EPCs)-mediated vasculogenesis, intussusception, tumor cell-endothelial cell vessel cooption, tumor cell dominant vasculogenic mimicry, and trans-differentiation of tumor cells into tumor endothelial cells [75]. Importantly, tumor cell-mediated neovascularization promotes tumor growth, progression, and cancer metastasis, and is closely associated with poor prognosis of cancer patients [76]. In light of that tumor cell-dominant vasculogenic mimicry plays an important role in tumor growth and metastasis, and contributes to poor prognosis of cancer patients, we recently investigated the effects of lycorine and other TCM herbs on tumor vasculogenic mimicry [74, 77, 78], and found that lycorine effectively suppressed metastatic melanoma cell-dominant vasculogenic mimicry, diminished melanoma cell-mediated formation of capillary-like tubes *in vitro* and generation of tumor blood vessels *in vivo* [11]. Mechanism study revealed that lycorine inhibits the activity of VE-cadherin promoter and markedly reduced VE-cadherin expression and cell surface exposure [11]. In addition, lycorine also impeded human ovarian cancer cell neovascularization through suppressing the formation of capillary-like tubes by Hey1B cells *in vitro* and the ovarian cancer cell-dominant vascularity *in vivo* when administered to Hey1B-xenotransplanted mice. In addition, lycorine diminishes the expression of several key angiogenic and vasculogenic genes, including VE-cadherin, vascular endothelial growth factor (VEGF), and semaphorin 4D (Sema4D). Furthermore, lycorine reduces Akt phosphorylation and blocked Akt signaling pathway in Hey1B cells (Figure 3) [12]. Collectively, lycorine effectively inhibits tumor neovascularization.

3.3 Anti-virus of lycorine

Lycorine has a selective anti-virus effect on HIV-1 virus [13], severe acute respiratory syndrome-associated coronavirus (SARS-CoV) [14], poliovirus [15], flaviviruses [16], human enterovirus 71 (EV71) [17], and avian influenza virus H5N1 [18]; but not inhibits several other viruses, such as alphavirus (Western equine encephalitis virus) and rhabdovirus (vesicular stomatitis virus) [16].

Mechanism studies show that lycorine blocks the viral RNA replication and suppresses the viral protein synthesis [18]. Lycorine strongly hinders RNA synthesis in flaviviruses and weakly inhibits viral protein translation. A gene allele substitution of viral 2K peptide in lycorine-resistant West Nile virus (WNV) confers the virus resistance to lycorine via an enhancement of viral RNA replication [16, 18]. Lycorine also inhibits the incorporation of $^{[3H]}$leucine into poliovirus-infected HeLa cells and partially abolishes protein translation in the cells in a dose-dependent manner, implying that lycorine may impede protein synthesis in virus infected cells [79]. Lycorine blocks the protein synthesis of the 3′-terminal region of the polyvirus translation unit, but not affect the protein synthesis of either 5′-terminal or middle region of the polyvirus translation unit [79]. In addition, lycorine can completely protect the mice from pathologic symptoms upon a low-dose EV71 virus challenge through blockade of virus polyprotein elongation during translation [17]. Ribonucleoprotein (RNP) complex plays a pivotal role in the viral generation and replication. Amaryllidaceae alkaloids do not affect the activity of the RNP complex, however, Amaryllidaceae alkaloid hemanthamine can suppress the migration of RNP from nuclear to cytoplasm, and lycorine delays the export of nucleoprotein protein from the nucleus [18]. Collectively, lycorine is a potential candidate drug for anti-virus therapy.

3.4 Anti-bacteria of lycorine

Lycorine inhibits growth of several *Saccharomyces cerevisiae* strains rho+, rho-, and mit-; whereas the strains devoid of mitochondrial DNA are resistant to lycorine at concentration of more than 200 μg/mL, suggesting mitochondrial genes are involved in the anti-bacterial effect of lycorine [19, 20].

Mechanism studies indicate that the resistance of the rho0 stains to lycorine is related to the deletion of RTG gene, a key mitochondrial functional gene [21]. Lycorine effectively inhibits growth of isogenic rho+ RTG and rho0 Drtg strains, but not affect the growth of isogenic rho0 strain with RTG nuclear genotype. In addition, the isogenic rho0 strains with deletion of RTG genes are sensitive to lycorine. The structure-activity relationship studies show that the aromatization of the C ring and the oxidation to a C-7 azomethine group of the B ring are structural features for anti-bacterial function of lycorine [80]. Besides, the position of the oxygenation of the C ring and the presence of the 1,3-dioxole ring join to the A ring of the pyrrolo [de]phenanthridine skeleton in lycorine, and these rings play a pivotal role in anti-bacterial activity of lycorine (Figure 1).

3.5 Anti-inflammation of lycorine

Lycorine and its derivative lycoricidinol (nacriclasine, which is shown in Figure 1) possess anti-arthritis activity in

various arthritis animal models [22–25]. Lycorine effectively blocks rat paw edema induced by carrageenan with an ED_{50} of 0.514 mg/kg [24]. Calprotectin is a pro-inflammatory factor and contributes to the development of inflammation. When the inflammation exists *in vivo*, the extracellular calprotectin is greatly increased [81], resulting in tissue destruction in severe inflammatory diseases. Lycorine and its derivative lycoricidinol effectively diminish calprotectin-induced cytotoxicity [81]. Besides, lycoricidinol significantly reduces the degree of swelling of both adjuvant-treated and untreated feet [81]. Lycorine also blocks lipopolysaccharide (LPS)-induced production of pro-inflammatory mediators and decreases LPS-induced mortality in mice [25]. Mechanistical studies showed that lycorine curbs the LPS-induced up-regulation of inducible nitric oxide synthase and cyclooxygenase-2 protein levels in RAW264.7 cells. In addition, lycorine suppresses the release of nitric oxide, PGE2, TNF-alpha, and IL-6 from LPS-treated RAW264.7 cells, and inhibits LPS-induced activation of P38 MAPK and Jak-STAT signaling pathways. Together, the unique anti-inflammatory function of lycorine makes it as a new drug candidate for anti-arthritis and anti inflammation therapies.

3.6 Inhibition of acetylcholinesterase and topoisomerase activities by lycorine

Alzheimer's disease (AD) is a degenerative, progressive, and irreversible disorder that causes cognitive impairment and intellectual dysfunction. The patients with AD exhibit brain cholinergic dysfunction, notably, a decrease in acetylcholine (ACh) level leads to difficulties with memory and attention, resulting in brain cholinergic dysfunction. Acetylcholinesterase (AChE) is the enzyme which degrades Ach; hence, suppression of AChE can protect brain ACh from degradation, providing a novel strategy for the AD therapy. Up to now, four AChE inhibitors have been used in the clinics to treat the AD patients at mild to moderate disease stages. However, these AChE inhibitors display a low efficacy in anti-AD therapy; the development of effective drugs for AD patients is in great demand. Although lycorine shows a relatively weak AChE inhibitory activity, a lycorine type alkaloid, assoanine (Figure 1), is the most active AChE inhibitor among the lycorine family alkaloids, with a four fold lower IC_{50} than that of an anti-AD drug galanthamine used in the clinics [26, 27]. The structure and function studies indicate that an aromatic ring C is critical to a certain planarity and hydrophobic interaction and plays key role in binding of the lycorine type compounds to AChE to reduce AChE activity (Figure 1) [82].

Lycorine also shows significant inhibitory effect on DNA topoisomerase I activity that is required for cancer cell growth [28]. Lycorine is the only one with great topoisomerase I inhibitory activity among several pyrrolophenanthridine alkaloids using genetically engineered mutants of the yeast *Saccharomyces cerevisiae* strains RAD+, RAD52Y, and RS321. In view of that topoisomerase inhibitor, such as doxorubicin, has been widely used in the clinics to treat various cancers, lycorine and derivatives may be good topoisomerase inhibitors for novel anti-cancer drug discovery.

CYP3A4, a family member of cytochromes P450, is the main isoenzyme responsible for the drug metabolism. A screen of CYP3A4 inhibitors showed that narciclasine (lycoricidinol) and silylated lycorine analogues of the lycorine type alkaloids significantly inhibited CYP3A4 activity; whereas, the crinane and galanthamine types in amaryllidaceae alkaloids did not possess any inhibitory effect [29]. In addition, structural study reveals that the C1–C10b double bond and lipophilic substitution at C2 of P3A4 account for lycorine analogues-mediated inhibition of CYP3A4 activity. These data provide new insight into the structure and function of lycorine analogues for the further drug design and development.

3.7 Suppression of ascorbic acid biosynthesis by lycorine

Ascorbic acid is synthesized in plants from glucose and acts as a free-radical scavenger in a variety of cell compartments [32]. Ascorbic acid is also a common antioxidant component in the apoplast and is known to be a strong *in vitro* inhibitor of peroxidases. Lycorine has been proved to be an inhibitor of ascorbic acid biosynthesis *in vitro* and *in vivo* [32]. Lycorine strongly inhibits the *in vivo* conversion of galactono-gamma-lactone to ascorbic acid and likely functions as a non-competitive inhibitor of galactono-gamma-lactone oxidase through forming a stable bound with the enzyme [33]. In fact, lycorine at a dose of 50 μM rapidly exerted a strong inhibitory effect on the ascorbic acid biosynthesis, and the effect persisted even if the alkaloid had been removed from the incubation medium. Further studies showed that lycorine selectively inhibited the activity of L-galactono-gamma-lactone dehydrogenase, but did not affect the activities of ascorbate peroxidase, ascorbate free radical reductase, and dehydroascorbate reductase [33]. As an inhibitor of the last enzyme of the ascorbic acid de novo biosynthetic pathway, lycorine may be applied in multiple areas, such as plant breeding and plant culture [34, 35].

3.8 Other functions of lycorine

In addition to the above multiple biological functions, lycorine was found to be the most potent alkaloids against *Plasmodium falciparum* [6]. Analysis of the chemical structure and anti-malarial activity of lycorine show that the best anti-plasmodial effect is achieved with lycorine derivatives that present free hydroxyl groups at C-1 and C-2, or esterified as acetates or isobutyrates. Additionally, the double bond C-2-C-3 also plays an important role in the anti-

plasmodial effect of lycorine derivatives (Figure 1).

Trichomonosis is the most prevalent non-viral sexually transmitted disease. The patients suffered from the infection by *Trichomonas vaginalis* in urogenital tract [37]. *Trichomonas vaginalis* was characterized by ecto-nucleotidases such as nucleoside triphosphate diphosphohydrolase (NTPDase) and ecto-5′-nucleotidase. Lycorine and candimine significantly abolish the NTPDase and ecto-5′-nucleotidase activities of the parasites, while the transcript levels of NTPDase A or B are not affected [37]. Further study shows that lycorine causes the *Trichomonas vaginalis* cell cycle arrest without affecting cell apoptosis [38]. In addition, lycorine shows a strong aphicidal activity to other parasites such as *Tribolium castaneum* and *Aphis gossypii* [39].

Furthermore, lycorine can control circadian period length [40]. When cells were pretreated with lycorine and then the agent was removed, the period length was reverted as compared with that of the control cells, indicating that elongation of the circadian period induced by lycorine and lycoricidinol is reversible. Although lycorine inhibits protein synthesis in several studies mentioned above, the period elongation by lycorine is in a cycloheximide independent manner, and cycloheximide itself do not affect period length, suggesting that lycorine extends the circadian period by a mechanism other than inhibition of protein synthesis. Moreover, lycorine was found to modulate transcription of the key clock gene named brain and muscle Arnt-like protein-1 (Bmal1), resulting in the circadian period changes. These data suggest that lycorine and its derivatives are novel regulators which control circadian period length.

Over and above, lycorine was also found to have a choleretic effect in a rat model [83], and to exert multiple biological functions, such as body temperature-lowering action, analgesia, and excitation of female animal's uterus [41].

4 Low toxicity of lycorine

Lycorine has trivial toxicity to normal cells and normal bodies. The animal toxicological experiments showed that the 50% lethal death rate (LD_{50}) of lycorine in mice was 112.2±0.024 mg/kg via intraperitoneal injection. The LD_{50} of lycorine in mice was 344 mg/kg via gastric lavage injection, suggesting that lycorine is very low toxicity in gastrointestinal administration [41]. Subcutaneous injection of 3mg/kg lycorine into beagle dogs only induced a short salivation increase, meanwhile, the dogs with the same dosage were observed to have mild continuous and paused vomiting and diarrhea [41]. In an open, prospective, randomized and controlled trial, subcutaneously administered lycorine induced nausea and emesis at 0.5 mg/kg body weight and reached statistical significance at 1.0 mg/kg, and the maximum emetic dose of lycorine (ED_{100}) was 2 mg/kg body weight [84]. Whereas, nausea and emesis were short-lasting and occurred not later than 2.5 h post dose. There is no other obvious side effect existing in the dogs, suggesting lycorine is low toxicity. Interestingly, it has been reported that lycorine had low toxic effect *in vivo* dog model [85]. The experiment from a various antiemetic drugs on lycorine-induced nausea and emesis *in vivo* in dogs showed that predominantly neurokinin-1 and to a lesser extent 5-hydroxytryptamine 3 receptors were involved in lycorine-induced emesis [86].

In vivo studies showed that lycorine had linear plasma kinetics with a mean elimination half-life of 0.67 and 0.3 h after single *s.c.* and *i.v.* administration, respectively. The mean oral bioavailability was calculated to be about 40%. Biochemical and hematological parameters of safety studies indicate no pathological signs.

Liu *et al.* showed that lycorine inhibited the proliferation of five human leukemia cell lines in a dose-dependent manner, with the IC_{50} values ranging from 1.5 to 5.5 μM, while the viability of the normal peripheral blood mononuclear cells from five healthy subjects was not significantly affected by the same treatment at lycorine concentrations of up to 50 μM. The sensitivity of cancer cells to lycorine is 15-fold higher than that of corresponding nonmalignant cells [8, 11, 12]. Moreover, it has been recently reported that lycorine reduced carbon tetrachloride-induced hepatotoxicity, normalized the significant decreases of glutathione, vitamin C content, and inhibited the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and GSH reductase, indicating that lycorine exerts an anti-toxic effect [24]. Collectively, lycorine exerts its numerous pharmacological effects on various diseases with very low toxicity.

5 Conclusion remarks and perspectives

Lycorine and its derivates have divergent chemical structure and multiple biological functions and pharmacological effects, including anti-tumor, anti-tumor neovascularization, anti-virus, anti-bacterial, anti-inflammatory, anti-parasite, anti-plasmodia, and inhibition of ascorbic acid biosynthesis (Figure 3). Despite of the sound progress in lycorine studies, there are several issues need to be solved. First, the divergent structure of lycorine and its derivatives relevant to their functions is still incompletely understood. Hence, further study of the relationship between structure and function will bring new insight into the multiple biological and pharmacological effects of lycorine, provide guidance to design new compounds based on these lycorine structure-function studies, and create more potent lycorine-derivers for novel drug discovery. Second, the mechanisms of lycorine-mediated anti-tumor, anti-virus, and many other effects are still unclear. Therefore, it is highly desired to further investigate the mechanism of lycorine-mediated pharmacological effects to further improve the efficacy of lycorine and deriva-

tives in the clinics to treat diseases. Third, more pre-clinical and clinical studies are needed to further improve the efficacy of lycorine and derivatives in the treatment of various diseases. Taken together, the multiple biological functions and pharmacological effects on various diseases with very low toxicity of lycorine suggests that it is a potential drug candidate that warrants further investigation, and the translational studies of lycorine and derivatives will bring benefits to human beings.

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