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Anticancer, Anti-inflammatory, Analgesic, and Anxiolytic Effects of Koumine and Their Molecular Mechanisms

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Abstract Koumine is an indole alkaloid monomer extracted from the Chinese herb *Gelsemium elegans*, which has a variety of pharmacological effects. This paper provides a comprehensive summary of the pharmacological effects and molecular mechanisms of koumine, with a particular emphasis on its mechanisms of action in the context of anticancer, anti-inflammatory, analgesic, and anxiolytic properties. The aim is to provide a theoretical foundation for further research and the application of koumine in clinical practice.

Key words Koumine, Anticancer, Anti-inflammatory, Analgesic, Anxiolytic

1 Introduction

Gelsemium elegans (Gardn. & Champ.) Benth. is a plant of the genus *Gelsemium* in the family Loganiaceae, primarily distributed in Yunnan, Guizhou, Hunan, *etc.* The plant has been shown to have efficacy in the treatment of various ailments, including rheumatoid arthritis, anxiety, and malignant tumors. Its mechanisms of action include the dispelling of wind, the attacking of toxins, the subduing of swellings, and the relieving of pain[1-2]. Koumine (KM) is a significant active ingredient derived from the plant *G. elegans*. It is a white crystalline powder with a molecular formula of $C_{20}H_{22}N_2O$ and a relative molecular weight of 306.407. It is soluble in organic solvents, including acetone, ether, and dimethyl sulfoxide. A substantial body of evidence from numerous studies has demonstrated that KM exhibits a diverse range of biological activities within the domain of biomedicine, encompassing anticancer, anti-inflammatory, analgesic, and analgesic properties[3]. The study offers a comprehensive review of the pharmacological effects of KM and its underlying molecular mechanisms, in order to serve as a foundation for further research and exploitation of KM.

2 Anticancer effect and molecular mechanism of KM

Cancer is a disease in which the organism, under the long-term joint action of various internal and external factors, undergoes uncontrolled proliferation of local tissue cells, which in turn results in the destruction of the structure and function of tissues and organs, and ultimately leads to the death of the patient due to organ failure. The advancement of medical technology has led to a notable improvement in the survival rate of cancer patients. However, the prevalent chemotherapeutic drugs have inherent limitations, including high toxicity, adverse effects, prolonged treatment duration, and high cost, which collectively compromise the quality of life and safety of patients. It has been demonstrated that KM exhibits advantageous properties, including low toxicity, high efficiency, safety, and affordability. It has been shown to induce apoptosis and inhibit the migration and invasion of cancer cells, thereby effectively reducing the vitality of a multitude of cancer cell types.

2.1 Anti-breast cancer effect and molecular mechanism of KM Zhang Xiaohua *et al.*[4] employed an MTT assay to ascertain the inhibitory effect of KM on the proliferation of human breast cancer MCF-7 cells. Their findings revealed that KM exhibited a dose-dependent inhibitory effect on MCF-7 cell proliferation, with an IC_{50} value of 124 μg/mL. The observation of morphological changes in the nuclei of MCF-7 cells by Hoechst 33342 nuclear staining revealed that cells treated with KM exhibited chromatin condensation, nuclear crumpling, enhanced fluorescence intensity, and nuclear fragmentation. Further investigation of the cycle arrest and apoptosis-inducing effects of KM on MCF-7 cells by flow cytometry and protein immunoblotting assay demonstrated that KM blocked the MCF-7 cell cycle at the G_2/M phase and up-regulated the expression levels of pro-apoptotic proteins Bax and Caspase-3, while down-regulating the expression level of the anti-apoptotic protein B lymphoblastoma-2 (Bcl-2) expression level, which in turn induced apoptosis in MCF-7 cells. The aforementioned results demonstrated that KM has the potential to impede the proliferation of the human breast cancer cell line MCF-7 at the G_2/M phase of the cell cycle and induce apoptosis in these cells by regulating the Bcl-2/Caspase-3 signaling pathway.

2.2 Antihepatocellular carcinoma effect of KM and its molecular mechanism Yuan Zhihang *et al.*[5] employed an MTT assay and flow cytometry to investigate the impact of KM on the viability and induction of apoptosis in human hepatocellular carcino-

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ma (HCC) cells. Their findings revealed that KM enhanced the apoptosis rate of HCC cells in a dose-dependent manner, thereby inhibiting the proliferation of these cells. The impact of KM on the expression levels of apoptosis-related proteins in HCC cells was evaluated through a protein immunoblotting assay, which demonstrated that KM elevated the expression of Bax and cleaved Caspase3 proteins while reducing the expression of Bcl-2 and Procaspase3, thereby inducting apoptosis in HCC cells. The alterations in mitochondrial membrane potential and their impact on ROS accumulation were evaluated through flow cytometry. The findings revealed that KM effectively diminished mitochondrial membrane potential and augmented ROS deposition in HCC cells. The reversal of this phenomenon was significantly influenced by the addition of ROS inhibitors. Furthermore, to elucidate the molecular mechanism of KM-induced apoptosis in HCC cells, alterations in the expression levels of pertinent signaling pathway proteins were examined through protein immunoblotting assay. The results demonstrated that KM diminished the expression levels of p-ERK, p-p38, p-p65, and p-I-κBα proteins. The addition of ROS inhibitors resulted in a notable reversal of the phosphorylation levels of ERK, p38, p65, and I-κBα proteins. The aforementioned findings indicate that KM suppresses the activation of the ERK/p38 MAPK and NF-κB signaling pathways by enhancing the expression of ROS in HCC cells, thereby inducing apoptosis in HCC cells.

2.3 Anti-colonic adenocarcinoma effects of KM and its molecular mechanism Chi Debiao *et al.* [6] employed acridine orange staining to identify apoptosis in human colon adenocarcinoma LoVo cells, and their findings indicated that KM induced apoptosis in LoVo cells in a time-dependent manner. Further detection of cycle block by KM on LoVo cells by flow cytometry revealed that after 24 h of treatment with KM, the rate of cell cycle arrest at the G_0/G_1 phase increased from 31.3% to 42.3%, while the rate of S-phase cells decreased from 62.0% to 38.7%. The aforementioned results demonstrate that KM has the capacity to impede the cycle of LoVo cells at the G_0/G_1 phase, thereby inducing apoptosis of LoVo cells.

2.4 Anti-rectal cancer effects of KM and its molecular mechanism Wang Lin *et al.* [7] constructed a protein-protein interaction network (PPI) to identify the potential active components and targets of KM with anti-rectal cancer effects. Additionally, they conducted a GO enrichment analysis and performed molecular docking to validate their findings. A total of seven compounds were identified as having docking results, among which KM demonstrated optimal spatial orientation within the active site, superior binding affinity to pyruvate dehydrogenase kinase (PDK1), and the formation of hydrogen bonds with the backbone of Lys111 and Glu130, which are situated within the structural domain of the kinase. Furthermore, the kinase activity assay corroborated the potential inhibitory effect of KM on the activity of PDK1 kinase. The inhibitory effect of KM on the proliferation of human rectal cancer cells was detected by MTT assay. It was found that KM could effectively inhibit the proliferation of HCT116 and HT29 cells with IC_{50} values of 70.56 and 62.82 μmol/L, respectively. Furthermore, the apoptosis-inducing effect of KM on HCT116 and HT29 cells and its underlying molecular mechanism were investigated through the use of Annexin V-FITC/PI double staining, flow cytometry, and protein immunoblotting assays. The results demonstrated that the apoptosis rate of human rectal cancer HCT116 and HT29 cells was significantly elevated from 8.1% to 33.0% following treatment with varying concentrations of KM. KM was observed to upregulate the expression levels of cytochrome C (Cyto C), Bax, and Caspase-3, while simultaneously downregulating the expression level of Bcl-2 in HCT116 and HT29 cells, thereby inducing apoptosis in HCT116 and HT29 cells. The aforementioned results suggest that KM can induce apoptosis in rectal cancer cells by regulating the expression level of apoptosis-related proteins, thereby inhibiting the proliferation of rectal cancer cells.

3 Anti-inflammatory effects of KM and its molecular mechanism

Inflammation represents a fundamental pathological process, initiated by living tissues with a vascular system, in response to a variety of external stimuli. While inflammation is typically beneficial to the organism as a defense mechanism against harmful stimuli, there are instances where it can be detrimental. These include attacks on the body's own tissues, inflammation occurring in transparent tissues, and other similar occurrences that can significantly impact human health. It has been demonstrated that KM exerts beneficial anti-inflammatory effects and can be employed in the treatment of numerous inflammatory disorders.

Luo Yufei *et al.* [8] employed enzyme-linked immunosorbent assay (ELISA) to detect the secretion of inflammatory cytokines in BMDM and THP-1 macrophages. Their findings revealed that lipopolysaccharide (LPS) and three activators (ATP, nigericin, and monosodium urate crystal) all stimulated BMDM macrophages to promote interleukin-1β (IL-1β) secretion in the supernatant of the cultures. The secretion of IL-1β in BMDM macrophages was markedly diminished following pretreatment with KM and MCC 950, an inhibitor of the NOD-like receptor protein 3 (NLRP3) inflammasome. The MTT and CCK-8 assays were employed to assess the toxicity of KM on macrophages. The results demonstrated that KM did not exert any significant toxic effects on macrophages. The inhibitory effect of KM on the activation of NLRP3 inflammasome in the peritonitis mouse model in vivo was further investigated through the use of flow cytometry and ELISA assay. The results demonstrated that KM effectively attenuated neutrophil recruitment and inhibited IL-1β production in mice with peritonitis. The inhibitory effect of KM on NLRP3 inflammasome activation and its mechanism were further investigated through protein immunoblotting, immunofluorescence staining, and real-time fluorescence quantitative PCR (RT-qPCR) assays. The results demonstrated that, in the context of KM treatment in conjunction with BMDM stimulation via LPS and a range of NLRP3 inflammasome activa-

tors, there was a notable decline in NLRP3, pro-IL-1β protein expression levels and mRNA levels. Additionally, the nuclear localization of I-κBα, p65 phosphorylation, and p65 exhibited a substantial reduction. Subsequently, it was demonstrated that KM markedly suppressed the secretion of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) in macrophages stimulated with LPS and ATP, as evidenced by an ELISA assay. Furthermore, the detection of reactive oxygen species (ROS) levels in BMDM and THP-1 macrophages by flow cytometry demonstrated that KM markedly diminished ROS levels in macrophages generated by LPS and ATP treatment. The aforementioned results indicate that KM suppresses the activation of NLRP3 inflammasome by modulating the ROS/NF-κB signaling pathway, which in turn inhibits the secretion of inflammatory cytokines in macrophages.

Yuan Zhihang *et al.*[9] investigated the impact of KM on the protein expression level of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production in LPS-induced RAW264.7 macrophages through a protein immunoblotting assay with a Griess method. The findings demonstrated that KM markedly reduced the expression of iNOS protein in LPS-stimulated RAW264.7 macrophages, concurrently diminishing NO production. Furthermore, the use of ELISA to ascertain the impact of KM on LPS-induced secretion of diverse inflammatory cytokines in RAW264.7 macrophages demonstrated that KM potently suppressed the generation of IL-1β, IL-6, and TNF-α in LPS-stimulated RAW264.7 macrophages. Furthermore, the impact of KM on the expression levels of NF-κB and MAPK signaling pathway-related proteins in LPS-stimulated RAW264.7 macrophages was evaluated through a protein immunoblotting assay. The results demonstrated that KM markedly diminished the LPS-induced activation of the NF-κB signaling pathway and the phosphorylation of IκBα, p65, p38, and ERK[10]. The aforementioned results indicate that KM diminishes the synthesis of pro-inflammatory mediators, thereby attenuating the inflammatory response. This is achieved by inhibiting the phosphorylation of p38 and ERK and by suppressing the activation of the NF-κB signaling pathway in LPS-stimulated RAW264.7 macrophages.

4 Analgesic effect of KM and its molecular mechanism

Bone cancer pain is a symptom of pain resulting from the invasion of bone tissue caused by the tension of a tumor on the periosteum or the pressure exerted on blood vessels, nerves, and surrounding tissues. In recent years, the advent of novel therapeutic modalities, including neoadjuvant chemotherapy and targeted drug therapy, has led to a notable improvement in the remission rate of cancer pain patients. However, despite these advances, the therapeutic landscape for bone cancer pain remains constrained, with a significant proportion of patients experiencing suboptimal control of their pain. The administration of KM was observed to result in a reduction of bone cancer pain in rats.

Zhang Wenjie[11] extracted spinal cord tissue proteins at the lumbar enlargement of mice by establishing a bone cancer pain (BCP) model and a sham surgery (Sham) model. The activation of microglia was then detected by protein immunoblotting assay and immunofluorescence. The results demonstrated a notable elevation in protein expression levels of p-PI3K, p-Akt, Iba1, IL-6, and IL-1β in the spinal cord of the BCP group in comparison to the Sham group. Additionally, the immunofluorescence-positive areas of p-PI3K, p-Akt, and Iba1 in the spinal cord dorsal horn (SDH) of the mice were all significantly increased. In contrast, the intrathecal injection of PI3K inhibitor LY294002 significantly reversed the expression level of related proteins and the size of the immunofluorescence-positive area, thereby alleviating BCP in mice. The analgesic effect of KM on BCP mice was investigated further through the use of pain behavioral assay, protein immunoblotting assay, immunofluorescence assay, and pathological histological staining. The findings indicated that the paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) were markedly elevated in KM-treated mice. Conversely, the protein expression levels of p-PI3K, p-Akt, Iba1, IL-6, and IL-1β exhibited a notable decline, while the immunofluorescence-positive area of p-PI3K, p-Akt, and Iba1 demonstrated a substantial reduction in the SDH of mice. The aforementioned results indicate that KM has the potential to mitigate BCP by inhibiting the activation of the PI3K/AKT signaling pathway, attenuating microglia activation, and reducing the release of inflammatory factors.

5 Anxiolytic effects of KM and its molecular mechanism

Anxiety, also known as anxiety neurosis, can be categorized into two forms: chronic anxiety and acute anxiety. The latter is often accompanied by autonomic and motor symptoms such as dizziness, chest tightness, palpitations, shortness of breath, tremor, and so on. It is a neuropsychiatric disorder with high morbidity. It has been demonstrated that KM exerts a pronounced anxiolytic effect.

Zhong Zhifeng[12] investigated the impact of KM on the anxiety-like behavior of mice using the functional observational battery (FOB) test. The findings indicated that KM markedly diminished the manifestation of anxiety-like behaviors, including grasping, head-touching, and panic, in mice. The open field test (OFT) and the Vogel conflict test (VCT) demonstrated that mice treated with KM exhibited no alterations in movement distance and average speed throughout the open field. Conversely, the percentage of movement distance, the percentage of dwell time, and the number of entries in the central area were significantly increased. Additionally, the number of licks, licking time, and number of electroshocks were significantly elevated in KM-treated rats in the VCT test. A rodent pathological anxiety-like model was further established by the sound evoked by natural enemies, and the plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone were measured to investigate the antipathological anxiety effect of

KM and its mechanism of action. The findings revealed that KM markedly elevated the levels of pregnenolone and allopregnenolone in the prefrontal cortex, hippocampus, and amygdala, while concurrently reducing the blood concentrations of ACTH and corticosterone in rats exhibiting the natural enemy sound-induced anxiety-like model. The aforementioned results indicate that KM exerts anxiolytic effects by modulating the levels of progesterone and allopregnenolone in the brain and regulating the activity of the hypothalamic-pituitary-adrenal (HPA) axis.

Huang Huihui *et al.* [13] investigated the anxiolytic properties of KM by utilizing an elevated plus maze (EPM) in rats and mice. Their findings revealed that, following acute administration of mice and continuous administration of rats in EPM, KM markedly elevated the percentage of open arm entries (OE%) and open arm time (OT%) in the EPM. However, KM did not influence the locomotor activity of rats and mice in the EPM. Furthermore, solid-phase extraction in conjunction with high-performance liquid chromatography-mass spectrometry (HPLC-MS) was employed to ascertain the concentration of neurosteroids within the hippocampus of rats, with the objective of investigating the anxiolytic impact of KM and its influence on the neurosteroid level within the hippocampus. The findings revealed that KM elevated the levels of the neurosteroids pregnenolone and allopregnenolone within the hippocampus. The aforementioned results indicate that KM exerts anxiolytic effects by increasing the levels of the neurosteroids pregnenolone and allopregnenolone in the hippocampus. Notably, the effective dose is considerably lower than the median lethal dose (LD_{50}), exhibiting a high degree of safety. Consequently, KM is expected to be developed into a novel type of high-efficiency and low-toxicity anxiolytic drug.

6 Prospects

G. elegans is documented in the *Compendium of Materia Medica* and numerous other pharmacological monographs. KM represents the primary bioactive component of *G. elegans*. KM exhibits a multitude of pharmacological activities, including anticancer, anti-inflammatory, analgesic, and anxiolytic effects. It boasts several advantageous properties, such as low toxicity, high efficiency, a vast range of sources, and can be utilized as a starting raw material for the synthesis of a plethora of products with medicinal value and crucial pharmaceutical intermediates. Nevertheless, the study of the pharmacological effects of KM is still in its infancy. It is imperative to continue to comprehensively and systematically study the chemical composition and molecular mechanism of the pharmacological effects of KM, and to further explore potential pharmacological effects, in order to provide a theoretical basis for the further development and utilization of KM.

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