**Activities of Naturally Occurring Alkaloids Bulleyaconitine A**

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**Introduction**

Bulleyaconitine A with C19-diterpenoid alkaloid exhibited various bioactivities, including anti-inflammatory, analgesic activity, immunomodulatory effects, and toxicities (Figures 1). In 1980, bulleyaconitine A was firstly isolated from a folk herbal medicine *Aconitum bulleyanum* Diels, which was used for the treatment of chronic pain and rheumatoid arthritis in west Yunnan province by Kunming Institute of Botany, Chinese Academy of Science. In 1980s, bulleyaconitine A has been approved by the China Food and Drug Administration as intramuscular injections, tablets and soft gel capsules for treating chronic pain, and rheumatoid arthritis in China. To date, its dosage forms have extended to oral liquid, patches, capsules for treating chronic pain, and rheumatoid arthritis in China. The structure of bulleyaconitine A.

![Figure 1](image)

**Bioactivities and relevant mechanisms of bulleyaconitine A**

**Antinociceptive activity**

For centuries, the Aconitum genus has been used for analgesic, antirheumatic and neurological indications in China and other Asian countries, in which bulleyaconitine A accompanied with other alkaloids, such as mesaconitine, 3-acetylaconitine, and lappaconitine, play an important role. It was initially claimed that the antinociceptive activity of bulleyaconitine A might have some relationship with the voltage-gated sodium channel because it can reduce neuronal Na+ current in a use-dependent manner and display long-acting local anesthetic properties in rats. However, according to the result of King’s lab recently, bulleyaconitine A actually performs its antinociceptive activity by stimulating the dynorphin A expression in spinal microglia and performs its anti-hypersensitivity in the same way. Dynorphin is known as an analgesic biomaterial produced by the precursor protein prodynorphin expressed in spinal microglia, and it has been proved to have a strong antinociceptive activity. Surprisingly, bulleyaconitine A dose contribute to the expression of prodynorphin in spinal microglia by activating the cAMP-PKA-p38β-CREB signaling pathway. In this pathway, the Gs-protein, cAMP/ PKA, p38β MAPK as well as CREB, are successively activated in the spinal microglia one by one through a series of phosphorylation, which mediates prodynorphin expression, and finally performs its antinociceptive activity and anti-hypersensitivity.

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Anti-inflammatory activity

In 1983, Tang firstly reported bulleyaconitine A showed anti-inflammatory activity.[5] In 1996, the result of a clinical study on 134 patients, in which 53 and 30 patients struggled with rheumatic arthritis and rheumatoid arthritis, respectively, showed that the application of bulleyaconitine A could relieve or even eliminate their clinical symptoms and its total effectiveness comes up to 93.3%.[14] Besides, the combination therapies of bulleyaconitine A applied in many cases presented better anti-inflammatory or antinociceptive effects than the single application of bulleyaconitine A. For example, bulleyaconitine A combing with chondroitin sulfate was applied in the clinical treatment of periarthritid of shoulder in 1995.[15] In 2005, bulleyaconitine A combing with high dose Danshen (salvia miltiorrhiza) was treated for clinical cancer pain.[16] Bulleyaconitine A combing with intrarticular injection of sodium hyaluronate or Shuangbai powder or tripterygium glycosides tables was cured knee osteoarthritis in 2013 and 2015, respectively.[17,19] In 2014, bulleyaconitine A combing with methotrexate was used for the treatment of rheumatoid arthritis.

However, the mechanism of its anti-inflammatory is unclear. In 2013, the project of “Effect of Bulleyaconitine A on Adjuvant Arthritis Rats” was conducted by Long Li’s group for investigating the therapeutic effect of bulleyaconitine A on adjuvant arthritis and exploring the treatment mechanism. The result[20] showed that 100 μg/kg of bulleyaconitine A can reduce significantly the circumference of arthritic ankle in adjuvant arthritis Wistar rats. Moreover, the level of TNF-α and PGE2 in serum decreased in 100 μg/kg bulleyaconitine A treated rats (p<0.01), and the level of TNF-α in serum also decreased in 50 μg/kg bulleyaconitine A group, which suggested that bulleyaconitine A can improve the inflammation of adjuvant arthritis Wistar rats by reducing the level of TNF-α and PGE2 in serum.

Immunomodulatory effects

Bulleyaconitine A used in rheumatoid arthritis (RA), possesses not only antinociceptivity and anti-inflammation, but also immunomodulatory effects.[13,14] RA is known as a long-term autoimmune disorder which involves the body’s immune system attacking the joints. Bulleyaconitine A can relieve rheumatoid arthritis through its immunomodulatory effects according to Ye’s report.[21] Considering that both the specific and non-specific immune responses are involved in the formation of arthritis, bulleyaconitine A was used as a whole administration for observing its effect on some immune functions in Balb/c mice. Balb/c mice were divided randomly into three groups including physiological saline group as a control group, bulleyaconitine A groups (0.08, 0.16, 0.32 mg/kg, intramuscularly) as test groups, and hydrocortisone (10 mg/kg) group as a positive control group. Mice were sacrificed after administration for 7 d, and relevant evaluation indexes, such as spleen-index, thymus-index, lymphocytes proliferation, level of total IgG in serum, phagocytosis of peritoneal macrophages (Mφ), NO in supernatants of macrophages, were tested and recorded by different methods. Bulleyaconitine A (0.32 mg/kg) inhibited T- and B-lymphocytes proliferations and reduced NO in supernatants, bulleyaconitine A (0.16, 0.32 mg/kg) decreased the thymus-index and the level of total IgG in serum, and bulleyaconitine A (0.08, 0.16, 0.32 mg/kg) inhibited the phagocytosis of peritoneal macrophages (Mφ). The result indicates that bulleyaconitine A has a suppressive effect on some immune functions of Balb/c mice.

Toxicities of bulleyaconitine A

Bulleyaconitine A also has some toxicities despite its strong antinociceptivity and approval of China Food and Drug Administration. The toxicities of bulleyaconitine A have always been concerned since 1980s, and many research on it has been done to show its clinical safety. In 1986, a toxicologic research on mice and rats was conducted by Tang’s group. Both mice and rats were injected poisoning-dose bulleyaconitine A, whose values are 10–40 times as the clinical application dose. After injection, toxic symptoms appeared, including salivation, vomiting, continuing convulsions, respiratory depression, and even the development of asphyxia death. Dead ones were within 12 h after administration and survivors were recovered after 24 h.[13] Wang et al. found that bulleyaconitine A can induce acute systemic toxicity in rats, including hyporeceptivity, arrhythmia, sedation, and respiratory distress. They also made some attempts to reduce this kind of systemic toxicity by co-administration of lidocaine or epinephrine and it did work. Finally, it was concluded that the systemic toxicity of bulleyaconitine A could be minimized by reducing blood flow at the injected site.[3] There are also many side effects in clinical research, such as dizziness, nausea, general sensation, palpitations, numbness, body itching and bilateral lower extremity urticaria, sweating, dry mouth, injection site swelling, allergic reaction, distinct feeling of pain at injection site, etc. But they were tolerable and would disappear after some time.[4,22] It is possible that the biological activity of bulleyaconitine A may be caused by its toxicity rather than a specific pharmacological action.[23] However, King’s group found that the anti-hypersensitivity of bulleyaconitine A can be separated from its neurotoxicity by the mechanism of action,[24] and the relatively wide therapeutic window of bulleyaconitine A suggests that its antinociception could be separated from its toxicity.[13] It means that bulleyaconitine A performs its ability by a specific pharmacological action in some way rather than its toxicity.

Metabolites and metabolic pathways of bulleyaconitine A

The metabolites of drugs are always being concerned, and they cause the side effects or perform its toxicities. The research on the metabolites and the metabolic pathways of bulleyaconitine A plays an important role during the period of explaining the bioactivities and toxicities of bulleyaconitine A. In 2013, the metabolites and the metabolic pathways of bulleyaconitine A in rat liver microsomes were investigated by Bi’s group, twelve metabolites of bulleyaconitine A were identified and some important CYP isoforms contributing to the metabolism of bulleyaconitine A were discovered. Bulleyaconitine A was added into the prepared microsomal suspension containing NADPH and specific CYP inhibitors (α-naphthoflavone for CYP1A2, quinidine for CYP2D, diethyldithiocarbamate for CYP2E, ketoconazole for CYP3A and sulfaphenazole for CYP2C), then acetoneitrile was added after the incubation of the suspension to stop the reaction.[25] Finally, the supernatant was analyzed using HPLC-MS after centrifuging for some time. The results are shown in Figure 2 and Table 1.

**Figure 2** The structures metabolites of bulleyaconitine A.

The results indicated that bulleyaconitine A is mainly metabolized by CPY3A and CPY2C in rat microsomes. Moreover, reactions happened on its side chains, including demethylation, de-ethyllyation, hydroxylation, didemethylation, deacetylation and dehydration.
Determination and quantification of bulleyaconitine A in vivo

Liquid chromatography and mass spectrometry play an important role in the determination and quantification of bulleyaconitine A in vivo. The extreme low concentration of bulleyaconitine A for its low dose and rapid metabolism results in great difficulties for developing the analytical methods. Before 2004, none of reported methods for the determination of bulleyaconitine A in blood or urine is validated, and mass spectrometry detection (GC-MS) and gas chromatography including high-performance liquid chromatography with UV or sensitive enough for the pharmacokinetic study in humans. Before 2004, none of reported methods for determination of bulleyaconitine A in blood or urine is validated.

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