

Research progress on pharmacological actions and molecular mechanisms of Anemoside B4

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Abstract

Anemoside B4 (AB4), as an important ingredient of Chinese herb *Pulsatilla Chinensis*, has been used in Chinese ancient medicine for thousands of years with exact therapeutic effects of anti-inflammation and anti-tumor. Due to its high potency and low toxicity, AB4 has caused more and more attention of researchers. In this paper, we will first review the clinical usage and fundamental research of AB4 against damaging inflammation of liver, lung, kidney, intestine and joints. Then, we will overview its application and molecular mechanisms in a range of cancers, such as liver cancer, lung cancer, colorectal cancer and cervical cancer. In addition, we pay attention to its pharmacological actions and molecular mechanisms against other diseases, for example, retinal injury, allergic conjunctivitis, pain, vulvovaginal inflammatory disease, acute infectious disease and oral ulcer. Finally, we will discuss the future perspectives of AB4, so as to improve its wide application in clinic.

1. Introduction

Pulsatilla Chinensis (Bge.) which belongs to Ranunculaceae, is originally recorded in the book of Shennong's Classic of Materia Medica. According to Traditional Chinese Medicine theory, the dry root of the herb is bitter and cold in nature, and regulates the stomach and large intestine meridian, thus, can be used for the treatment of heat toxin, blood dysentery, vaginal pruritus, and increased leucorrhea [1-3]. According to the above mentioned indications, *Pulsatilla* is used in the treatment of acute and chronic inflammation, cancer, schistosomiasis and amoebic dysentery [4]. A number of patent prescriptions contain *Pulsatilla*, such as Pulsatilla decoction, Compound Pulsatilla Colon Tablets, Compound baitouweng colon targeting tablet, Changtai mixture, and Baipuhuang tablets. The main ingredients of *Pulsatilla* are triterpenoid saponins, triterpenoid acids and small molecular components lignans, coumarins, and sterols.

As the abundant and active component among the triterpenoid saponins in herbal medicine, thus, Anemoside B4 (AB4) is regarded as a Q-marker for the *Pulsatilla Chinensis* [5, 6]. Current reports and researches indicating that AB4's major pharmacological actions including anti-inflammatory, anti-tumor, analgesic, anti-virus and antibacterial [7]. At present, studies mainly focus on its anti-inflammatory and anti-tumor activities. This paper principally overviews the research progress on the pharmacological actions and molecular mechanisms of AB4 against inflammation, tumor, and other diseases. In detail, we will first review the clinical usage and fundamental research of AB4 against damaging inflammation of liver, lung, kidney, intestine and joints. Then, we will overview its application and molecular mechanisms in a range of cancers, such as liver cancer, lung cancer, colorectal cancer and cervical cancer. In addition, we pay attention to its pharmacological action and molecular mechanisms against other diseases, for example, retinal injury, allergic conjunctivitis, pain, vulvovaginal inflammatory disease, acute infectious disease and oral ulcer. The detailed studies of AB4 on multiple diseases are summarized in Table 1 and Figure 1, 2. Finally, we will discuss the future perspectives of AB4, so as to improve its wide application in clinic.

Table 1. Pharmacological actions of AB4 on different disease models.

Pharmacological actions	Disease models	Expressions of factors	Refs
Anti-inflammatory mechanism of AB4 (Suppressed cell apoptosis, inflammatory response and oxidative stress; enhanced cell autophagy)	Acute liver injury: carbon tetrachloride-induced mice/sepsis in CLP-induced mouse model and LPS-induced primary hepatocytes	serum TBIL, AST, ALT, TNF- α , IL-6 and IL-1 β ↓; CAT, SOD and GSH activities↑	[8, 9]
	Acute lung injury: LPS-induced mice/LPS plus ATP-induced THP-1 cells/ sepsis in CLP-induced mice /LPS plus BLM-induced mice	neutrophil↓; serum TNF- α , IL-6, IL-1 β and MPO↓; iNOS and COX-2↓	[10, 11]
	Pneumonia: KP- and FM1-induced mice	MPO, TNF- α , IL-1 β and IL-6↓	[12]
	COPD: Cigarette smoke exposure combined with LPS mice and rats	MMP-2, MMP-12, TIMP-1↓; MMP9↑; T-bet, STAT4 and IL-12↓; GATA3, IL-4 and STAT6↑; IL-2, IL-5, IL-18, IL-10, TGF- β , TNF- α and IL-1 β ↓	[13, 14]
	Pulmonary fibrosis: BLM-induced mice	IL-4, TGF- β 1, TNF- α ↓	[15]
	Acute renal injury: Cisplatin-induced mice	BUN and Crea↓; p53, Bax and cleaved-caspase-3↓; Bcl-2/Bax↑; TNF- α , IL-1 β , COX-2 and iNOS↓	[16-18]
	Chronic kidney injury: Adenine-induced rats	BUN, Crea and urine protein↓; IL-6, IL-1 β , NF κ B, NLRP3, Caspase 1, ASC and IL-18↓; podocin and nephrin↑	[19]
	Chronic renal failure: Adenine-induced rats	BUN, Scr and MDA↓; SOD↑; IL-1 β , IL-6 and TNF- α ↓; Bax and caspase-3↓; p-Akt and Bcl-2↑	[20]
	Ulcerative colitis: 8% acetic acid-induced rats/DSS-induced mice/TNBS-induced rats	p53, cleaved-caspase-3 and Bax↓; Bcl-2↑; IL-1 β , IL-6 and TNF- α ↓	[21-23]
	Gouty arthritis: MSU-induced THP-1 cells	NALP3, Caspase-1 and ASC↓; TNF- α , IL-1 β and PGE ₂ ↓	[24, 25]
	Chronic endometritis: 25% phenol mucilage induced rats	IL-6, gp130 and STAT3↓	[26]
	Ischemia-reperfusion injury: Removing right kidney and ligating left kidney	NLRP3, ASC and caspase-1↓; IL-1 β , IL-18, TNF- α , IL-6, TLR4 and NF- κ B↓	[27]

Antitumor mechanism of AB4 (Induced cell apoptosis and autophagy)	Liver cancer: Huh-7/SMMC7721 /HepG2 cells	Caspase-3↑; Belin-1↑; LC3 and p62↓	[28-30]
	Lung cancer: NCI-H460 cells	Ras, ERK1/2, GLUT1 and MCT4↓	[31]
	Colorectal cancer: LoVo/L-OHP cells/HCT116 and 5-FU cells	P-gp↓; caspase-9↑	[32, 33]
	Cervical cancer: HeLa cells	Beclin1 and Atg5↑	[34]
Mechanism of AB4 in treating other diseases	Diabetic retinopathy: STZ-induced rats	Bax and Caspase-3↓; Bcl-2↑	[35, 36]
	Allergic conjunctivitis: Ovalbumin-induced rats	p-JNK and p-p38↓	[37]
	Pain: Hot plate, thermal radiation, Von Frey cilia mechanical stimulation, acetic acid writhing and formaldehyde experiment-induced mouse pain model	mechanical pain threshold↑; writhing↓; foot licking↓	[38]
	Vulvovaginal candidiasis: HL60 cells/Candida albicans resistant strains-induced rabbits	F-actin↓	[39, 40]
	Hand foot mouth disease: EV71-infected RD cells	14-3-3 protein↑; IL-10 and IFN-γ↑	[41, 42]
	Oral ulcer: acetic acid and paclitaxel-induced rats	healing time↓	[43]

2. Anti-inflammatory mechanisms of AB4

Inflammation is the body's immediate defense response of living tissues and cells to injury factors such as physical injury, chemicals and pathogens under stimulated conditions [44]. The central link of its process is vascular response, which is mainly manifested in redness, swelling, heat, pain and dysfunction. Dozens of clinical studies and experiments have proved many representative Chinese medicines and their derivatives have anti-inflammatory effects by regulating TLR-4/MyD88/NF-κB, PI3K/Akt/mTOR and JAK/STAT 3 signaling pathways [45]. Similarly, the mechanisms of AB4 inhibits apoptosis, oxidative stress and enhance autophagy against inflammatory diseases including damaging inflammation of liver, lung and kidney were reported.

2.1 Liver injury

The liver is the largest parenchymal organ of the human body, with major functions of metabolism, detoxification, secretion, and excretion of bile, as well as regulating two systems of human coagulation and anti-coagulation. However, liver is fragile and should be easily damaged by autoimmune

abnormalities, alcohol, fat, drugs, infection, and trauma [46]. Yang [8] discovered that AB4 showed significant protective effect on liver injury by reducing the contents of TBIL, AST, ALT, TNF- α , IL-6, IL-1 β in serum, increasing the contents of GSH-Px and SOD in liver tissue on the base of CCl₄ induced acute liver injury mice model. Its hepatoprotective effect may be achieved by inhibiting inflammatory response and antioxidant stress mediated by TLR-4/NF- κ B signaling pathway. Moreover, based on the CLP led mice sepsis model and LPS induced acute liver injury model, Pei [9] found AB4 could reduce serum AST, ALT, TNF- α , IL-6, IL-1 β levels and increase CAT, SOD and GSH activities, reduce the pathological damage of liver tissue, and increased the expression of autophagy-related proteins including Beclin-1, LC3II/LC3I, as well as reduced the content of p62 and promote the formation of autophagic vesicles in the liver. The above evidence suggested that AB4 could inhibit apoptosis, inflammation, oxidative stress and enhance autophagy, and improve acute liver injury through regulating mTOR/p70S6K-mediated autophagy pathway.

2.2 Pulmonary inflammation

Acute lung injury (ALI), as a respiratory tract disease with very high rates of morbidity and mortality, is characterized by recruitment of pulmonary neutrophils and macrophages, destruction of alveolar endothelial cells, and diffuse injury of alveolar capillaries with main clinical manifestations including respiratory distress and refractory hypoxemia. Critically ill patients even develop life-threatening acute respiratory distress syndrome (ARDS) [47]. Zhou [10] found that AB4 significantly reduced neutrophils amount, TNF- α , IL-6 serum levels in the lung and NF- κ B protein level in the LPS-induced ALI mice model. Yuan [11] built the *in vitro* macrophage THP-1 inflammatory model induced by LPS and ATP, discovered that after administration of AB4, the activation of NLRPP3 inflammatory body significantly reduced the expression of iNOS and COX-2, inhibited TNF- α , IL-6, IL-1 β and MPO, as well as other inflammatory factors, at the same time protected MMP. In addition, AB4 destroyed the dimerization of TLR4, regulated the activation of NLRP3 inflammatory bodies, and improved lung injury. In mice with sepsis, AB4 (5,10mg/kg) significantly improved the survival rate of mice (80%), as well as alleviated lung injury and reduced the release of inflammatory cytokines. AB4 (2.5mg/kg) showed a significant protective effect on LPS-induced septic death. It was also observed that AB4 showed a protective effect in the ALI mice model. In conclusion, AB4 might be regarded as the potential drug for the treatment of ALI, by blocking the dimerization of TLR4 and the activation of NLRP3 inflammatory bodies.

Pneumonia refers to the inflammation of lung parenchyma including terminal airways, alveolar spaces, and pulmonary interstitium, with the most common etiology of pathogenic microorganisms infection, physical and chemical factors, immune damage, and drugs[48]. Most of the clinical manifestations are cough and dyspnea accompanied with fever. In severe cases, it will even develop into lung abscess, acute lung injury, and hypoxemia. Pneumonia is also the leading cause of infective death among children worldwide. In the study of He [12], AB4 was found to reverse the increased number of NEU and WBC in mice with bacterial pneumonia, also inhibit MPO, TNF- α , IL-1 β and IL-6, and significantly inhibited the expression of TLR4, MyD88 and MD-2 induced by FM1. Thus, AB4 might reduce lung injury by inhibiting the activation of the TLR4/MyD88 signaling pathway.

Chronic obstructive pulmonary disease (COPD) is an irreversible disease characterized by airflow limitation with main causes including smoking, fuel fumes, air pollution, and respiratory infections. In the elderly, the incidence rate and the mortality rate of COPD is high, thus, COPD has become one of the most important chronic non-communicable diseases that threaten the health of residents [49]. Based on COPD rat model, Wang [13] found that AB4 significantly reduced the release of protease such as MMP-2, MMP-12 and TIMP-1 the gene and protein expression of the transcription factor T-bet, IL-12 and STAT4, and increased the expression of transcription factors GATA3 and IL-4 in the rat lung tissue. The above findings indicating that AB4 inhibited IL-12/STAT4 signaling pathway in Th1 cells and activated the IL-4/STAT6 signaling pathway in Th2 cells to achieve the anti-inflammatory effect. Ma [14] also found that AB4 decreased inflammatory factors IL-2, IL-5, IL-18, IL-10, TGF- β , TNF- α , and IL-1 β , significantly reduced the gene expression levels of MMP2, MMP12 and TIMP1, whereas increased that of MMP9. It could improve oxidative stress by balancing the expression of protease and anti-protease and inhibit precancerous lesions by up-regulating p53 and down-regulating FHIT expression.

Pulmonary fibrosis is the end-stage change of most lung diseases that has fewer treatment options, which is characterized by fibroblast proliferation and accumulation of a large number of extracellular matrices [50]. Respiratory function of most patients with pulmonary fibrosis was seriously affect manifested as dry cough and progressive dyspnea. Pulmonary fibrosis is progressive and irreversible even led to death, which the average survival time got definite diagnosis is only 2.8 years, and the 5-year survival rate is less than 30% [51]. Yang [15] found that AB4 decreased inflammatory factors related to pulmonary fibrosis including IL-4, TGF- β 1 and TNF- α , reduced the level of neutrophils and protected pulmonary fibrosis.

2.3 Renal injury

Cisplatin is a commonly used anti-tumor chemotherapy drug in clinic. However, due to the non-specificity, its anticancer activity is directly proportional to its concentration with side effects such as nephrotoxicity [52]. Based on the cisplatin-induced acute renal injury model in mice, Li [16] found that AB4 could prominently reduce the content of BUN and Crea in serum, and reduce the shedding and degeneration of renal tubular epithelial cells, and reduce pathological damage such as renal tubular protein casts. In addition, AB4 even reversed the up-regulated apoptosis-related protein expression (p53, Bax, and cleaved-caspase-3 proteins), suggesting that it could activate p53, repair damaged cells, promote anti-apoptosis and thereby play a role in the prevention and treatment of AKI. According to the results of Wang [17], AB4 could be regarded as a potential renal protective agent, which inhibited the NF- κ B signaling pathway, reduced TNF- α , IL-1 β , COX-2, and iNOS levels, activated MAPK signaling pathway and its downstream apoptotic factors, up- and down-regulated apoptosis-related proteins such as Bcl-2, Bax, caspase-3, and caspase-9. In another research of chronic kidney injury, Gong [19] discovered that AB4 mainly prevented glomeruli induced by adenine through suppressing the expression of inflammatory cytokines (IL-6, IL-1 β , IL-18, etc.) and increasing the content of two major podocyte proteins (podocin and nephrin). He [18] found that AB4 effectively increased the activity of HEK-293T cells treated with cisplatin, inhibited apoptosis, reduced the content of ROS, and increased the activity of SOD *in vitro*. Whereas, the

in vivo results showed that AB4 could regulate body weight and renal index, reduce the increased BUN and Crea levels of cisplatin, and improve renal histopathological injury.

Guo [20] found that the administration of AB4 could decrease the levels of BUN, Scr and MDA in serum, increase SOD activity, reduce oxidative stress response, phosphorylate PI3K/Akt pathway and reduce contents of IL-1 β , IL-6, and TNF- α based on a rat model of CRF induced by adenine. With the release of inflammatory factors, the expression of Bax and caspase-3 pro-apoptotic proteins was decreased, and the expression of p-Akt and Bcl-2 anti-apoptotic proteins was significantly increased, which is related to the effects of AB4 in reducing renal cell apoptosis and protecting renal tissue injury.

2.4 Intestinal inflammation

Ulcerative colitis (UC) is a chronic nonspecific inflammatory disease with unclear etiology, the lesions of which are limited to the mucosa and submucosa of the large intestine [53], mostly in the sigmoid colon and rectum. The course of UC is long and recurrent, whereas, there is no effective drug treatment for UC. Zhang [21] explored the effect of AB4 based on an acetic acid-induced UC rats model and found that AB4 could reverse the increase of p53, cleaved-caspase-3, Bax, and the decrease of Bcl-2, and reduce the levels of inflammatory factors IL-6 and TNF- α , which could inhibit apoptosis and inflammatory infiltration, and significantly protect and repair ulcers. Ma [22] established an UC mice model with dextran sodium sulfate (DSS) and found that AB4 could prevent the weight loss of UC mice, shorten the length of the colon, improve the pathological changes, and reduce the contents of IL-1 β , IL-6, and TNF- α in colon tissue. Meanwhile, AB4 might improve UC by inhibiting the activation of related factors in the TLR4 signaling pathway *in vivo* and decreasing the expression of key proteins in TLR4/NF- κ B/MAPK signaling pathway *in vitro*. Zhang [23] treated colitis rats induced by TNBS with AB4, and found that it improved the symptoms of colitis, inhibited the recruitment and activation of colonic neutrophils, and inhibited the expression of S100A9 and its downstream genes TLR4 and NF- κ B in the colon. *In vitro*, results demonstrated that AB4 could inhibit the NF- κ B signaling pathway induced by recombinant S100A9 Protein. In conclusion, AB4 inhibited the NF- κ B signaling pathway through deactivating S100A9 to prevent TNBS-induced colitis.

2.5 Joint inflammation

Gouty arthritis (GA) is a metabolic disease caused by uric acid in the joint capsule, bursa, cartilage, bone, and other tissues [54] with a high incidence rate in China and even in the global, which also seriously affects human health and daily life. Lv [24, 25] induced THP-1 cells with monosodium urate (MSU) crystals to mimic inflammatory conditions of GA, based on this model, AB4 was found to significantly improve GA by reducing NALP3 inflammatory body-related protein, inhibiting the biosynthesis of NALP3, caspase-1, and ASC, and reducing inflammatory cytokine TNF- α , IL-1 β , PGE₂.

2.6 Chronic endometritis

Chronic endometritis [55] (CE) is the local inflammation of the endometrium, the vast majority of which just show unspecific symptoms such as lower abdominal discomfort or increased leucorrhea. Wang [26] detected the protective effect of AB4 based on phenol mucilage induced endometritis model. As a result, the *in vivo* experiments showed that AB4 eliminated adhesion phenomenon in the uterine cavity of the uterus, improved the pathological structure of the uterus, decreased the gene and protein expression of IL-6 and STAT3 in uterus tissue. Whereas, the *in vitro* results indicating that AB4 significantly reduced the inflammatory factors IL-6, gp130, and STAT3 of LPS-induced rat endometrial epithelial cells. To sum up, the anti-inflammatory effect of AB4 was exerted by inhibiting IL-6/gp130/STAT3 signaling pathway.

2.7 Ischemia-reperfusion injury

Ischemia-reperfusion injury [56] (IR) refers to the situation that the degree of tissue injury increases rapidly after the ischemic tissue cells recover blood flow (reperfusion) for a certain time. After reperfusion, there is a large amount of Ca^{2+} influx and oxygen free radicals, which is the main pathogenesis of extensive tissue and cell injury. Based on the ischemia-reperfusion model, Li [27] clarified that AB4 could reduce the inflammatory factors related to renal NLRP3 pathway, including NLRP3, ASC, caspase-1, IL-1 β , IL-18, and the expression of inflammatory factors contained TLR4, NF- κ B, TNF- α , IL-6 in the TLR4/NF- κ B pathway. These findings suggest that AB4 could alleviate renal IR through regulating NLRP3 inflammasome and inhibiting TLR4/NF- κ B signaling pathway mediated inflammatory response.

3. Antitumor mechanisms of AB4

In recent years, AB4 has been reported to be effective towards various of tumor. Thus, we summerized the application of AB4 in the treatment of tumors (liver cancer, lung cancer, colorectal cancer and cervical cancer), and noticed that its core mechanisms might be regulating apoptosis and autophagy through PI3K/Akt/mTOR, Ras/MAPK, Caspase and Notch signaling pathways.

3.1 Liver cancer

Liver cancer is a common malignant tumor caused by hepatitis virus, aflatoxin, nitrosamine, and alcohol with major clinical manifestations including liver pain, abdominal distention, anorexia, and fatigue. The incidence rate of liver cancer is sixth among all cancers, whereas the mortality rate is fourth [57]. Xue [28] found out that AB4 down-regulated Bcl-2, up-regulated Bax, increased Bax/Bcl-2, activated caspase family (caspase-3), acted on PARP apoptotic protein, and then promoted the apoptosis of liver cancer cells. The pathology showed that the outline of tumor cells in the AB4 group was gradually blurred, with nuclear pyknosis, cytoplasm and fragmentation. Xue [29] also noticed that AB4 inhibited the proliferation of hepatoma SMMC7721 cells and induced apoptosis and autophagy by inhibiting the phosphorylation of Akt and mTOR. More interestingly, its combination application with chemotherapy drug PI3K/Akt/mTOR inhibitor was more effectively against cancer cells than that of the single application. As an autophagy-specific substrate, p62 interacts with LC3 to infiltrate autophagosomes and is efficiently degraded by autophagolysosomes. In AB4's treatment, LC3 flux and p62 degradation indicated it might induce cell autophagy, which was reversed by the autophagy inhibitor CQ. Wang [30] clarified that AB4 inhibited cell proliferation by regulating the HepG2 cycle and blocking G2/M phase replacement of human hepatoma

cells in vitro, and induced apoptosis by upregulating the expression of caspase-3. To sum up, AB4 could be used alone or in combination with chemotherapy drug to reduce the severity of liver cancer by inhibiting proliferation of cancer cells and activating apoptosis and autophagy.

3.2 Lung cancer

Lung cancer is one of the most common malignant tumors in the world, whose malignant degree and mortality rank first. With poor overall prognosis and low life quality, the approximately fewer than 20% of patients beyond 5 years from diagnosis [58]. Nowadays, the main treatments were surgery, radiotherapy, chemotherapy, and targeted therapy. Although the treatment technology is constantly improving, there are still tough problems needs to be solved, such as adverse reactions, drug resistance and side effects. Guan [31] found that the combination of active components of Pulsatilla saponins (A3, PSD, B4, etc.) could inhibit human lung cancer based on NCI-H460 cells by regulating the phosphorylation of Ras/MAPK and reducing the expression level of RAS, ERK1/2, GLUT1, and MCT4 oncoproteins. The above findings suggested that AB4 was potential to be in the combination treatment of Lung cancer.

3.3 Colorectal cancer

Colorectal cancer [59] is regarded as the third most common cause of cancer mortality worldwide. At present, chemotherapy is the most important treatment, however, chemoresistance [60] is an important cause for treatment failure. Li [32] found that the expression of P-gp protein (drug resistance protein) decreased after treatment with non-toxic dose of AB4, which could reverse the drug resistance of human colon cancer L-OHP resistant cells LoVo / L-OHP. He [33] also found that AB4 inhibited the proliferation of human colon cancer HCT116/5-FU cells in vitro and promoted the apoptosis of mouse drug-resistant colorectal cancer cells HCT11/5-FU in vivo. In addition, the combined treatment of AB4 and 5-FU induced the apoptosis of colorectal cancer cells by phosphorylation of Scr protein kinase and activation of caspase-9. In short, AB4 could be hopefully used alone or in the combination with other drugs in the treatment of colorectal cancer.

3.4 Cervical cancer

Cervical cancer, as the most common gynecological malignant tumor, is mainly caused by infection with high-risk human papillomavirus [61, 62]. Its mortality and incidence rate ranked fourth in the world, due to data calculated in 2018 [57]. Ren [34] found that AB4 could significantly inhibit the proliferation and migration of human cervical cancer HeLa cells by downregulating the Notch signaling pathway and promoting the expression of autophagy-related molecules Beclin1 and Atg5. To summarize, the above results give us a hint that AB4 might become an effective drug for the clinical treatment of cervical cancer with further research.

4. Mechanisms of AB4 in the treatments of other inflammatory diseases

4.1 Retinal injury

The retina is located in the inner layer of the eyewall and is a transparent film, which is composed of pigment epithelium layer and retinal sensory layer. The two layers can be separated under pathological conditions, which is called retinal detachment [63]. Niu [35] intraperitoneally injected AB4 to treat streptozotocin (STZ) induced diabetes in rats, and found that AB4 improved the diabetic retina structure and cell disorder effectively. At the same time, AB4 inhibited retinal cell apoptosis by up- and down-regulating apoptosis-related proteins (Bcl-2, Bax and Caspase-3) in rat retina. Besides, Liu [36] also found

that giving different concentrations of AB4 could reduce the incidence of cataracts induced by diabetes, from 66.67% to 30%, 25%, and 31.25% respectively. Taken together, the anti-retinopathy efficacy of AB4 was exerted through inhibiting cell apoptosis.

4.2 Allergic conjunctivitis

Allergic conjunctivitis is a common immunological hypersensitivity disease characterized by itching, redness, and swelling which is affecting up to 40% of the population especially during the allergy season [64]. Ma [37] constructed the ovalbumin-induced allergic conjunctivitis rat model and demonstrated that AB4 could reduce ocular signs and clinical scores, inhibit the structural changes of conjunctival tissue, alleviate the infiltration of eosinophils, and inhibit mast cell degranulation. Meanwhile, AB4 improved allergic conjunctivitis by inhibiting the expression of p-JNK and p-p38 proteins in the MAPK pathway. In conclusion, AB4 has a clear effect on the treatment of ocular changes caused by allergic conjunctivitis, which might be the important basis for anti-allergic conjunctivitis medicines.

4.3 Pain

As the basic symptom of many diseases, pain affects patients' life seriously. At present, anti-pain standard drugs used to relieve pain mainly includes opioids (fentanyl and tramadol), NSAIDs (ibuprofen and paracetamol), antidepressants (bupropion and duloxetine), antiepileptic drugs (gabapentin and lamotrigine), cannabinoids (cannabis), local anaesthetics (bupivacaine) and others (clonidine and ketamine) [65]. Different analgesic drugs have their relative merits, however, they also have own side effect such as insomnia, drowsiness, sexual dysfunction, emotional dullness and so on. Although they can alleviate pain to some extent, nearly 50% of patients needs further pain control strategies [66]. Take opioids and NSAIDs as an example. On one hand, opioids are greatly limited in application due to the addiction. On the other hand, the long-term and large-scale use of NSAIDs have serious adverse reactions. Therefore, the research and development of new analgesic drugs with high selectivity, good safety and low side-effects has become extremely urgent. Zhang [38] found that AB4 had analgesic effect based on several classic models, including hot plate, thermal radiation, Von Frey cilia mechanical stimulation, acetic acid writhing and formaldehyde experiment. In the hot plate, heat radiation tail-flick, and mechanical stimulation experiments, it was observed that AB4 administration markedly increased the mechanical pain threshold of mice. In the acetic acid writhing model, AB4 reduced the number of writhing. Based on the formaldehyde pain model, AB4 significantly reduced the time and times of foot licking in the second phase of mice, and markedly inhibit the formaldehyde induced inflammatory pain. To sum up, AB4 has obvious analgesic and anti-inflammatory effects, which could be expected to become a potential analgesic drug applying to the prevention and treatment of pain.

4.4 Vulvovaginal inflammatory disease

Vulvovaginal candidiasis (VVC) is a female genital tract fungal infection characterized by vulvar itching, burning pain, and dysuria. Although the occurrence of VVC is caused by *Candida albicans* infection, the degree of clinical manifestation of VVC patients is not directly proportional to the number of *Candida*

albicans in the vagina, but directly proportional to the number of neutrophils recruited into the vaginal cavity. At this time, neutrophils released a large number of inflammatory factors stimulated by *Candida albicans* rather than phagocytized *Candida albicans*, then, led to immune-inflammatory damage of vaginal mucosa and evolved into "pathogenic" neutrophils [67]. Therefore, targeting "pathogenic" neutrophils was an important strategy for the treatment of VVC. Based on HL60 cells, Yun [39] found that AB4 effectively inhibited the migration of neutrophils by reducing the expression of F-actin and inhibiting the PI3K/Akt pathway. Li [40] discovered that the combination of miconazole nitrate with AB4 showed better efficacy than that of the single use of AB4 in treating VVC based on a rabbit model. In short, AB4 synergized with other compound to enhance the anti-bacterial ability against VVC.

4.5 Acute infectious disease

Hand foot mouth disease (HFMD) is a viral infection commonly caused by Coxsackievirus A16 (CA-V16) and enterovirus 71 (EV-A71), which endangers children under 5 years old seriously. The major clinical manifestations are mouth sores, anorexia, low fever, or small red and painful spots on the hands, feet, and mouth [68, 69]. More importantly, there is no recognized specific therapeutics or prophylactics available so far. According to recent reports, in the model of EV71 infected RD cells (human rhabdomyosarcoma cell), Kang [41] found that AB4 up-regulated 14-3-3 (tyrosine 3-oxygenase) protein, destroyed the interaction between yes-associated protein, cancer-associated protein and interference regulator 3, and enhanced IFN- β reaction to resist the virus by activating RIG-I-like receptor pathway. In the model of EV71 infected suckling mice, it was found that AB4 reduced the complications of EV71 infection by inhibiting the replication of EV71, ameliorating muscle inflammation as well as limb activity, and regulating HFMD biomarkers IL-10 and IFN- γ . Moreover, Liu [42] also discovered AB4 could remarkably improve the muscle stiffness and survival rate of neonatal rats infected with EV71 by inhibiting the apoptosis and necrosis of RD cells. Collectively, the above results suggested that AB4 exerted the antiviral effect against HFMD by orchestrating Hippo and RLRs pathway.

4.6 Oral ulcer

The oral ulcer is a common ulcerative injury disease that occurs in the oral mucosa and causes extremely feeling of pain due to the massive distribution of nerves and blood vessels. The etiology and pathogenesis are not completely clarified so far, the treatment of oral ulcer is unsatisfied. According to the recent study, Liu [43] has proved that AB4 exerted therapeutic effect on oral ulcer with characters of reducing the ulcer healing time and improving the overall condition of rats including body weight and food intake. These results suggested that AB4 could be used to treat oral ulcer caused by chemotherapy drugs in tumor patients, so as to reduce the pain severity and improve their quality of life.

5. Future perspectives

In summary, a large number of studies have proved the anti-inflammatory, anti-tumor, antiviral and analgesic effects of AB4, however, the specific mechanisms remains to be studied. Based on our review, AB4 mainly affects TLR-4/NF-K β , PI3K/Akt/mTOR and JAK/STAT3 signaling pathways to inhibit the

inflammatory response of immune cells and protect liver, lung, kidney and intestine from inflammation dependent injury. According to published paper, AB4 induced apoptosis and autophagy of cancer cells through regulating PI3K/Akt/mTOR, RAS /MAPK, Caspase and Notch signaling pathways. In addition, researchers also found that AB4 has retinal protection, anti-allergic conjunctivitis, analgesia, antifungal and antiviral effects, but there are few relevant studies at present.

To sum up, the current pharmacological and toxicological studies of AB4 still remained in the basic stage, which might due to the limitation of traditional pharmacological methods. The above studies provided promising preclinical evidence of AB4 in the treatment of inflammation and cancer. However, with regard to pharmacological effects and molecular mechanism of AB4, prospective studies involving in-depth mechanistic research to reveal the unknown and novel signaling pathway and the molecular target(s) are of great urgency. Deciphering the molecular codes of AB4 will help to improve therapeutic strategies and extend its reasonable application.

Abbreviations

TCM: traditional Chinese medicine; CLP: cecal ligation and puncture; LPS: lipopolysaccharide; MMP: mitochondrial membrane potential; MyD88: myeloid differentiation protein-88; MD-2: myeloid differentiation protein-2; KP: Klebsiella pneumonia; FM1: influenza A virus FM1; CRF: chronic renal failure; TBIL: total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TNF- α : tumor necrosis factor- α ; TGF- β : transforming growth factor β ; IL-2: interleukin-2; IL-4: interleukin-4; IL-5: interleukin-5; IL-6: interleukin-6; IL-1 β : interleukin-1 β ; IL-10: interleukin-10; IL-18: interleukin-18; IL-12: interleukin-12; CAT: catalase; SOD: superoxide Dismutase; GSH: glutathione; MPO: myeloperoxidase; MDA: malondialdehyde; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase; MMP2: matrix metalloproteinase 2; MMP9: matrix metalloproteinase; MMP12: matrix metalloproteinase 12; TIMP-1: tissue inhibitor of metalloproteinase 1; T-bet: T-box expressed in T cells; STAT3: signal transducer and activator of transcription 3; STAT4: signal transducer and activator of transcription 4; STAT6: signal transducer and activator of transcription 6; BUN: blood urea nitrogen; Scr: Serum creatinine; NLRP3: Nucleotide binding oligomerization domain-like receptor thermal protein domain associated protein 3; ASC: adapter apoptosis-associated speck-like protein; p-Akt: phosphorylated-protein kinase B; PGE2: prostaglandin E2; gp130: glycoprotein-130; TLR4, Toll-like receptor 4; NF- κ B: nuclear factor kappa B; Ras: rat sarcoma; ERK1/2: extracellular regulated protein kinases; GLUT1: glucose transporter type 1; MCT4: monocarboxylate transporters 4; P-gp: P-glycoprotein; Atg5: autophagy-related gene 5; p-JNK: phospho-c-Jun N-terminal kinases; p-p38: phospho-p38 mitogen-activated protein kinases; 14-3-3: tyrosine 3-oxygenase; IFN- γ : interferon- γ ; CCl4: carbon tetrachloride; TNBS: 2,4,6-trinitrobenzene sulfonic acid.

Declarations

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Author contributions

QG and JW engaged in study design and coordination, material support for obtained funding, and supervised the review. TL, QW, HL and MZ performed literature research and wrote the manuscript. All authors reviewed and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interest.

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Figures

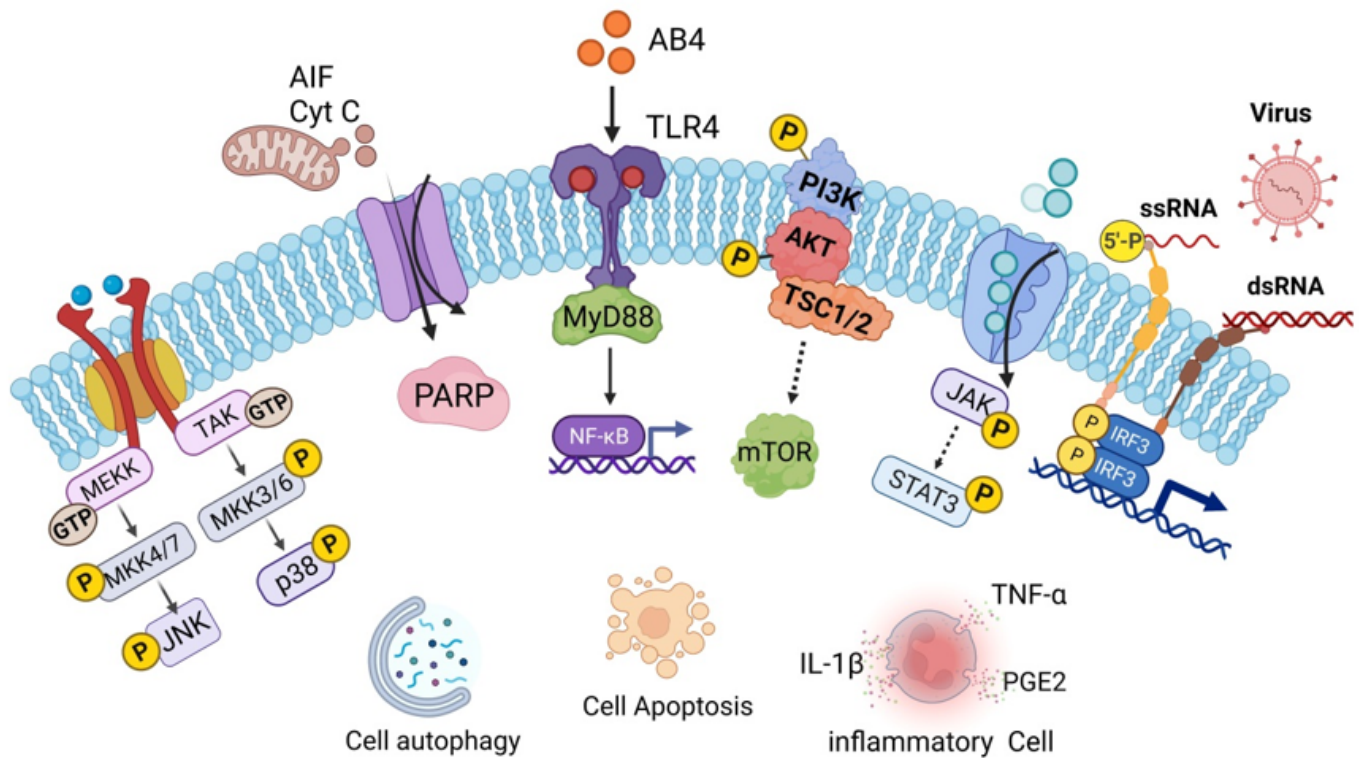


Figure 1

Molecular mechanisms of AB4 in the treatment of inflammation. AB4 suppressed inflammation mainly through inhibiting cell apoptosis, inflammatory response, oxidative stress and enhanced cell autophagy, which was further mediated by suppressing four signaling pathways, including Caspase, TLR4/MyD88, JAK/STAT3 and PI3K/Akt/mTORs.

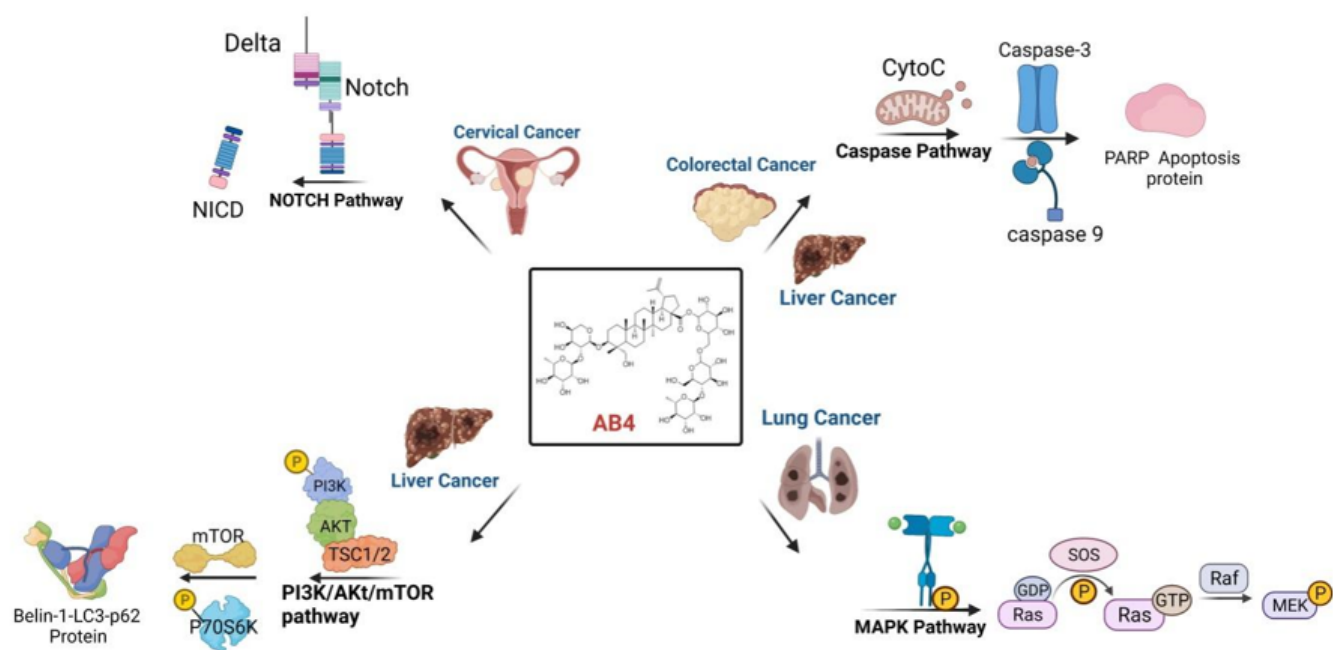


Figure 2

Pharmacological action of AB4 against multiple tumors. AB4 produced marked anti-tumor effect by inducing cell apoptosis and autophagy through inhibiting the Notch, Caspase, PI3K/Akt/mTOR and MAPK signaling pathways.