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Review

Phycocyanin: Anti-inflammatory effect and mechanism

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ABSTRACT

As the host defense response to various injuries and pathogens in the body, inflammation can remove damaged cells and pathogens in the host organism and protect the body. However, excessive inflammation may cause damage to normal tissue cells while removing pathogens, which in turn cause numerous inflammatory diseases and adversely affect the human health. Phycocyanin is an active substance extracted from *algae*; it has outstanding antioxidant and anti-inflammatory activities, and can effectively inhibit various diseases caused by inflammation. This review systematically summarizes recent applications of phycocyanin against various inflammatory diseases in lung, liver, cardiovascular, and cerebrovascular systems. In addition, possible anti-inflammatory action pathways of phycocyanin are reviewed to canvass the anti-inflammatory mechanism. At last, based on the existing research, phycocyanobilin in phycocyanin is proposed as a bilirubin analog by inducing heme oxygenase 1 in vivo to suppress inflammation.

1. Introduction

Inflammation is a dynamic feedback of the immune system response to internal or external damage and infection [1]. It is more like a chemical signal sent in the body to the immune system to promote the recovery and repair of damaged tissues, remove aged and diseased cells, and protect itself from foreign invaders of viruses and bacteria [2]. There are many factors that may induce inflammation. For example, the increased concentration of reactive oxygen species (ROS) in tissues, the increased cell apoptosis, or bacterial invasion can stimulate immune cells to secrete inflammatory factors and trigger inflammatory responses. When inflammation occurs, the blood flow of the tissue site raises, the permeability of the blood vessel increases, the immune cells (white blood cells or macrophages) are motivated through blood vessels to the injured spot, and release inflammatory factors [3]. This response do good to the body health by cleaning up damaged tissues and pathogens. However, when this process is unregulated, it would do harm to normal tissues with other serious diseases. However, the signaling pathways kin to inflammation are complex. For easy understanding, we divide inflammatory pathways into direct (such as toll-like receptors (TLR), nuclear factor kappa-B (NF-κB) pathway) and indirect pathways (such as oxidation and apoptosis pathways) according to their correlation with inflammation. In those signal pathways, the HO-1 gene in the Kelch-like ECH-associated protein 1 (Keap1)-NF-E2-related factor 2 (Nrf2) pathway that related to cellular oxidative damage aroused our interest. This gene encodes Heme oxygenases-1 (HO-1), an inducible enzyme that can decompose heme into biliverdin to play an antioxidant role [4]. Meanwhile, study on HO-1 demonstrates that it has anti-oxidation and anti-inflammatory activity [5–7], which makes HO-1 a significant target for inflammation treatment.

Algae has been widely consumed as an edible food and traditional Chinese medicine source since very ancient times. As early as AD 284–364, Ge Hong, a great ancient Chinese medical master, discovered a type of Nostoc and named it Gexianmi (*Nostoc sphaeroides*). Because Gexianmi (Gexian rice) has good nutrition and delicious taste, and is welcomed by the majority of people. At the same time, Gexianmi is rich in vitamins, phycobiliprotein, and other substances, which can play a role in regulating immunity and anti-inflammatory [8]. *Spirulina platensis*, like Gexianmi, belongs to cyanobacteria and is a low prokaryotic unicellular algae of Oscillatoriaceae. It is native to alkaline lakes in Mexico and Africa [9]. After being introduced into China in recent years, it has become a food supplement for medical function as a traditional Chinese medicine. Like other algae, including *Lyngbya*, *Trichodesmium*, *Porphyra* etc., *Spirulina* is recorded in the book "Chinese Ocean Materia Medica"[10], and its medicinal properties are described as "sweet, salty, and cool", and can boost functions of spleen and stomach, decrease blood lipids, ease the side effects from cancer radiotherapy and chemotherapy. Meanwhile, *Spirulina* is rich in nutrients. In addition to

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abundant vitamins, chlorophyll, and unsaturated fatty acids, it is rich in proteins, including algae specific light harvesting pigment protein phycobiliprotein [11]. Phycobiliprotein not only carries out photosynthesis, but also has many physiological functions, such as antioxidant [12] and anti-inflammatory [13] effects. Moreover, the cultivation of *Spirulina* is simple, and large-scale industrial production has been realized [14]. Therefore, *Spirulina* is expected to become a new star in food and drug industry.

Phycocyanin is a water-soluble light-harvesting protein from cyanobacteria, red algae, and some cryptomonads. The protein is composed of α and β subunits and is bound to phycocyanobilin (PCB) [15]. According to the different spectral properties, PC can be divided into R-PC and C-PC [16]. At present, industrialized phycocyanin products are mainly extracted from *Spirulina* [17], so the PC mentioned in this review is mostly C-PC. At present, studies on R-PC are relatively few, only a few studies reported anti-allergic, anti-inflammatory, and alleviating allergic airway inflammation activities [18,19]. In the latest research, the tetrapyrrole structure like bilirubin (BR) was believed to play an anti-inflammatory role in regulating the activity of immune cells in the body by inducing the expression of HO-1 and in reducing the inflammatory response [20,21]. In addition, phycobilin in tetrapyrrole structure was reported for featuring broad-spectrum inhibition against COVID-19 [22]. PCB bound to phycocyanin has a tetrapyrrole structure, which is similar to BR. With the structure, PCB is able to acquire similar antioxidant and anti-inflammatory activities as BR. The early anti-inflammatory study of PC can be traced back to 1998 when Romay et al. [23] found that PC can inhibit liver microsomal lipid peroxidation, mouse paw inflammation, and edema induced by glucose oxidase. Later, Romay et al.[24] perceived significant anti-inflammatory effect of PC in different inflammatory models, with which the release of histamine could be effectively reduced and the levels of myeloperoxidase and prostaglandin E-2 be minimized. Recent studies have revealed the anti-inflammatory activity of PC thoroughly and discovered that PC could act on NF-κB [25], TLR [26], and PI3K/Akt/mTRO pathway [27], thus directly inhibited the inflammation. In addition, PC can significantly up-regulate the expression of Nrf2, and indirectly inhibit tissue inflammatory response through antioxidant effect [28].

At present, although there have been sufficient studies on the application of PC in various inflammatory disease models, its possible targets are still unclear. Based on the summary of the role and signaling pathways of PC in different inflammatory diseases, in this article, we proposed possible anti-inflammatory targets of PC for precise application of PC.

2. The use of phycocyanin in inflammatory diseases

2.1. Inflammatory bowel disease

The intestines are not only the placement of nutrient intake and utilization, but also the congenital barrier of harmful substances such as pathogen and toxins from entering the body. Due to long-term exposure to the stimulation of pathogens and toxins, the intestines have become the main site of inflammation, and the abnormalities of intestinal barrier after inflammation is closely linked to the development of various diseases. PC is mainly digested in the gastrointestinal tract by oral intake, thus its anti-inflammatory activity can also take effect in the intestine to activate the anti-inflammatory effects. Therefore, studying the pathogenesis of intestinal inflammation would help in-depth research into the anti-inflammatory mechanism of phycocyanin.

For a long time, inflammatory bowel disease (IBD) has been considered a type of intestinal inflammation caused by the combination of the own genetics, external environment, and other factors [29,30].

Lu et al.[26] in 2020 used radiation-induced acute intestinal injury to mice to study the mechanism of PC in the prevention of intestinal inflammation. Results show that PC pretreatment (50 mg/kg) via month significantly improved the radioradiated intestinal damage caused by irradiation with 12 Gy X-ray. Meanwhile, PC could also regulate the composition of beneficial and harmful bacteria in the intestinal microbiota, thereby reducing lipopolysaccharide (LPS) levels, inhibiting the TLR4-Myd88-NF-κB pathway, and protecting against intestinal damage caused by excessive radiation. Moreover, Zhu et al. [31] studied mice colitis induced by 4.0% (w/V) dextran sodium sulfate (DSS). Results show that a daily intake of 150 mg/kg selenium-enriched PC (Se-PC) could effectively inhibit DSS-induced colitis, and supplementation of Se-PC could alleviate the weight loss and bloody diarrhea in the mice that suffered from colitis. This activity of Se-PC is thought capable of reducing the release of inflammatory factors in the gut, inhibiting the expression of NF-κB, and thus activating macrophages.

2.2. Atherosclerosis

Cardiovascular disease is an illness that seriously threatens human health. The disease has high morbidity, disability, and mortality, thus has become a major killer of human-being. The most representative of atherosclerosis in cardiovascular disease is confirmed to be related to inflammation and oxidative damage. The role of phycocyanin in arterial porridge hardening is discussed below.

Atherosclerosis (AS) is a chronic disease that consists of lipids and immune cells that accumulate on the walls of arteries. Additional to activating pro-inflammatory signaling pathways, AS is induced by further expression of cytokines and oxidative stress [32].

In 2013, Strasky et al. [33] examined how PCB could prevent AS from occurring, and they found that the tetrapyrrole structure of PCB could simulate the heme catabolic pathway products to protect atherosclerosis. Compared with the control, the 200 mM PCB-treated endothelial cells showed a 51% increase in HO-1 expression, and a 161% rise in HO-1 activity. In vivo experiments, by directly feeding *Spirulina platensis*, Strasky et al. [33] found that place with increased HO-1 expression was overlapped with the macrophage aggregation region. Compared with the control, *S. platensis*-treated mice showed a 27% increase in HO-1 expression in aorta and the activity of HO-1 promoter in spleen increased by 1.6 times. Quantitative stereological analysis showed that the taking-in *S. platensis* delayed the progression of atherosclerosis, and the atherosclerosis size in *S. platensis*-fed mice was reduced by about 12% against the control.

Both HO-1 and bilirubin can effectively inhibit the up-regulation of tumor necrosis factor (TNF)-α-induced endothelial dysfunction markers, such as E-selectin [34–36]. PCB bound in phycocyanin has a linear tetrapyrrole structure similar to that of bilirubin, and thus it may be similar to bilirubin in function and play antioxidant and anti-inflammatory roles. Results of Strasky et al. [33] show that PCB could play a similar role as bilirubin does and induce HO-1 expression against arteriosclerosis.

2.3. Liver inflammation

The liver contains a large number of active metabolic enzymes as the largest gland in human body. The abundant blood supply and unique structure make the liver's metabolic function highly active. It plays a key role in the metabolism of carbohydrates, fats, proteins, vitamins, and other substances, and it is an important organ for drug metabolism. Therefore, liver is among the most vulnerable organs.

2.3.1. Alcoholic liver disease

Alcoholic liver disease (ALD) is considered to be resulted from long-term alcohol abuse, and is one of the fatal diseases in the world. Every year, there are millions of ALD patients in the world, and 10% of the total deaths are ALD related [37]. When alcohol is ingested in human body, it is first metabolized in the liver after being absorbed through the gastrointestinal tract. On the one hand, alcohol in the liver can cause oxidative stress and cell apoptosis in liver tissue [38], and induce indirectly inflammation of liver tissue. On the other hand, alcohol can

directly induce Kupffe cells in the liver to activate and secrete inflammatory factors, resulting in liver tissue inflammation [39,40]. In the currently established ALD model, chronic alcohol intake induces oxidative stress and endotoxin sensitization, thereby activate the D14/TLR4 pathway and downstream signals, mediates the expression of inflammatory factors.

Xia et al. [41] fed female KM mice with 30% ethanol to induce symptoms of ALD, and studied the preventive effect of PC. They found that PC reduced alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood. The serum ALT and AST levels of the model group were increased by 113.6% and 96.72%, respectively, from those of the control. Compared to alcoholic liver mice, different doses of PC could reduce the levels of ALT and AST. Among them, the high-dose group of 400 mg/kg had the best inhibitory effect, with ALT inhibition rate of 36.50% and AST inhibition rate of 28.00%.

PC can also inhibit alcohol-induced subacute hepatocyte injury by improving the body's immunity. Compared with the model, PC can significantly improve the activity of $CD3^+$T and $CD4^+$T cells in serum, and increase the proliferation rate of T cells. After PC intervention, the proliferation of T cells and the $CD3^+$ and $CD4^+$T cells in liver injury mice were increased to different degrees, and the intervention effect of 400 mg/kg high-dose PC was the best [41].

2.3.2. Radiation-induced liver disease

Radiation-induced liver disease (RILD) is one of the serious complications of radiotherapy (RT). After a liver is irradiated with radiation, Kupffe cells, hepatic stellate cells, macrophages, and fibroblasts in the tissue would release a large number of cytokines (TNF-α, transforming growth factor-β (TGF-β) etc.) to participate in the early inflammatory process of RILD, and stimulate the differentiation of fibroblasts into myofibroblasts, eventually leading to the formation of radioactive liver fibrosis.

Liu et al. [28] in 2020 used 200 mg/kg PC to fedliver-damaged mice after X-ray irradiation and found that PC intervention could reduce the content of ROS in liver tissue. Among them, the effect of PC intervention was better than that of prevention, and returned to normal largely. After X-ray irradiation, the content of ROS in liver of the mice was increased, while the expressions of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) mRNA were decreased. SOD was just 25% and GSH-Px was 60 %, of the control group. Both PC prevention and intervention could restore the expression of peroxidase to normal and played an antioxidant role. Results show that PC could activate Nrf2 signaling pathway by upregulating the content of Nrf2 and HO-1 in liver tissue.

The above results confirm that PC can reduce the tissue damage caused by radiation by reducing oxidative stress. Under normal circumstances, Keap1 associated with Nrf2, resulting in Nrf2 ubiquitination and degradation. Therefore, in the absence of external stimuli, Nrf2 is usually maintained at low concentrations in cells. When there are stimuli such as oxidative stress in the body, Keap1 is inhibited, resulting in the continuous accumulation of Nrf2 in cells. In addition, in the promoter of the target gene, Nrf2 is able to bind to AU-rich element (ARE) on chromosomes, leading to the transcriptional induction of SOD, GSH-Px, and other cellular defense genes. As an important protective system of the body, the Keap1-Nrf2 pathway is widely involved in the damage of oxidative stress to the body, while PC can trigger the activation of this pathway and reduce the harm caused by radioactive liver injury [28].

2.4. Lung disease

The lung is responsible for the exchange of air between the animal body and the ambiance. The partial pressure of oxygen in lung is much higher than those in other organs. Because lung is always exposed to oxides, such as cigarette smoke, dust, and automobile exhausts etc., and it is the easiest to receive external stimuli, which in turn could cause inflammation of the lung tissue and damage the lung parenchyma [42]. Therefore, understanding the causes of pulmonary inflammatory diseases help us to use phycocyanin to intervene more accurately.

2.4.1. Acute lung injury

Acute lung injury (ALI) is a clinically critical emergency characterized by pulmonary diffusing dysfunction and has a high mortality rate. Although the exact mechanism of ALI is still unclear, it is generally believed that ALI is an uncontrolled inflammatory response mediated by pro-inflammatory mediators [43].

Phycocyanin has been demonstrated to improve acute lung injury through antioxidant and anti-inflammatory. In 2014, Zhang et al. [44] built a sepsis ALI model based on cecal ligation and puncture, and investigated the alleviating effect of PC on ALI by intervention with PC. Compared with the control, the content of TNF-α in bronchoalveolar lavage fluid (BALF) of the ALI mice increased from 12 to 428 pg/mL, and the interleukin (IL)− 1β and IL-6 increased from 2.46 and 3.18 pg/mL to 84.28 and 81.15 pg/mL, respectively. After PC (60 mg/kg) intervention, the IL-6, IL-1β, and TNF-α decreased to 24.34, 25.27, and 137.00 pg/mL, respectively, and the expression of HO-1 increased.

In addition, Cobalt (Co) protoporphyrin can up-regulate HO-1 gene expression and cooperate with PC to reduce the content of inflammatory factors, while the HO-1 inhibitor zinc (Zn) protoporphyrin can increase the level of inflammatory factors. The results show that PC may promote the body's resistance to oxidation and inflammation by up-regulating the expression of HO-1, and thereby reduce the acute lung injury caused by sepsis [44,45].

2.4.2. Pulmonary fibrosis

As an interstitial lung disease, pulmonary fibrosis (PF) is often accompanied by scarring of the lung tissue [46], damage to the alveolar structure, impaired lung gas exchange function, and ultimately lead to respiratory failure in the patients.

At the beginning, scientists used Paraquat (PQ), an organic pesticide, to induce pulmonary fibrosis in rats to study the therapeutic effect of PC on PQ-induced pulmonary fibrosis. Sun et al.[47] used 50 mg/kg PQ to induce acute pulmonary fibrosis in rats, and then took 50 mg/kg PC daily to treat pulmonary fibrosis, and the PC intervention reduced the expression of TGF-β1, thereby lessen the number of cells expressing NF-κB and TNF-α. The positive cells expressing NF-κB decreased from 6.69 % to 5.01 %, and the cells expressing TNF-α reduced from 13.23% to 7.83%.

As paraquat was completely banned in China in 2020, it is no longer appropriate to use paraquat to model pulmonary fibrosis rats. Therefore, scientists chose bleomycin that is widely used in cancer treatment, to model pulmonary fibrosis. Li et al. [48] in 2017 studied pulmonary fibrosis induced by bleomycin in rats. Through immunofluorescence and the Western blotting, after PC treatment, the levels of fibrosis-related protein vimentin, surfactant-related protein C, fibroblast-specific protein, and α smooth muscle actin decreased, in which the TLR2 pathway played an important role. After PC intervention, the expression of key genes in the TLR2 pathway was reduced. At the same time, $TRL2^{-/-}$ mice suffered less damage from bleomycin, but the effect of PC was also weaker. Experiments confirmed that TLR2-MyD88-NF-κB pathway is crucial in the process of PC reducing inflammatory factors and epithelial-mesenchymal transition to inhibit pulmonary fibrosis.

In addition, people also use X-rays to irradiate the lungs of mice to explore the ability of PC to reduce lung inflammation. A study shows that after X-ray irradiation, the concentrations of TNF-α and IL-6 in the lungs of mice increased significantly, reaching 350 and 85 μg/g prot, for which PC was used before or after irradiation [49]. PC reduced the syntheses of TLR2, Myd88, and NF-κB at the transcription and translation levels and lessened the inflammatory factors release, further verifying the role of TLR2- NF-κB pathway in PC anti-inflammatory and anti-pulmonary fibrosis.

Table 1
The biological roles of phycocyanin in different inflammatory diseases.

Source	Type	Target	Mechanism	Ref.
		Inflammatory bowel disease		
Arthrospira platensis	PC	Gut microbes	By affecting the TLR4/NF-κB pathway through gut microbiota, PC has a protective effect on the intestinal injury of mice caused by high-dose radiation.	[26]
		Atherosclerosis		
Spirulina platensis	C-PC	CD59	C-PC promotes the expression of CD59 gene in mice, delay or inhibit the occurrence and development of atherosclerosis.	[60, 61]
Spirulina platensis	PCB	HO-1	PCB regulates tissue oxidative stress and endothelial dysfunction substance levels by activating HO-1 expression.	[33]
Unknown	C-PC	LDL receptor	C-PC is positively correlated with the expression of Low-density lipoprotein (LDL) receptor mRNA, and C-PC has a lipid-lowering effect.	[62]
		Alzheimer's disease		
Leptolyngbya sp. N62DM	C-PC	β-secretase	Phycocyanin αβ-dimer interacts with β-secretase to inhibit the formation of amyloid precursor protein.	[63]
Unknown	C-PC	AS3T α-synuclein	By inhibiting AS3T α-synuclein, C-PC prevents the formation of fibrils and protects AD.	[52]
Unknown	PC		By preventing the increases in hippocampal cholinesterase and BAX activity, and the increases in BCL-2 and ChAT levels, PC can increase the levels of BDNF and IGF-1 and reduce neuroinflammation.	[53]
		Ischemic brain injury		
Unknown	PCB	HO-1	PCB can restore the expression levels of MBP and CNPase in ischemic rats, and promote the expression of HO-1.	[55]
Unknown	PCB	Mal/Bcl-2a1	PCB can down-regulate cytokine levels in striatum and serum and induce antioxidant gene expression, thus alleviating BCCAo-induced oxidative stress.	[56]
		Multiple sclerosis		
Unknown	PCB		PCB reduces the expression of inflammatory factors in the brain and relieves multiple sclerosis.	[64]
		Non-alcoholic fatty liver		
Spirulina platensis	C-PC	NF-κB	C-PC inhibits the NF-κB pathway and reduces the ratio of lymphocyte surface antigen ($CD4^+/CD8^+$).	[65]
Spirulina platensis	C-PC	AMPK	C-PC up-regulates AMPK phosphorylation and the expression of transcription factor peroxisome proliferators-activated receptors (PPAR) α, and down-regulates Sterol-regulatory element binding proteins (SREBP)− 1c level.	[66]
		Alcoholic liver		
Arthrospira platensis	C-PC	T Cell	C-PC promotes the proliferation of serum T cells, inhibits the levels of serum ALT, AST, MDA, etc., and increase the expression of SOD in the liver.	[41]
		Radiation liver injury		
Spirulina platensis	C-PC	Nrf2	C-PC reduces radiation-induced oxidative stress damage and reduces radiation-induced DNA damage by activating the Nrf2/HO-1 signaling pathway.	[28]
		Acute lung injury		
Unknown	C-PC	HO-1	By up-regulating the expression of HO-1, C-PC restores the pulmonary gas exchange function of acute lung injury and reduces tissue inflammation.	[44, 45]
Unknown	C-PC	NF-κB	By reducing the relative content of TNF-α, Malondialdehyde (MDA) and NF-κB, C-PC inhibits paraquat-induced lung injury.	[67]
Unknown	C-PC	NF-κB/ caspase-3 and Bax	By reducing peroxidase activity, ROS formation, and activation of NF-κB, C-PC regulates the content of apoptotic protein in lung tissue, and prevents LPS-induced acute lung injury.	[43]
		Pulmonary Fibrosis		
Spirulina platensis	PC	Gut microbes	PC reduces the number of bacteria associated with inflammation, significantly increases the number of short-chain fatty acids (SCFAs)-producing bacteria and probiotics. In addition, PC reduces the expression of inflammatory factors induced by bleomycin, and inhibits pulmonary fibrosis.	[68, 69]
Spirulina platensis	C-PC	NF-κB p65	C-PC decreases TGF-β1 protein content and NF-κB p65, TNF-α activity.	[47]
Spirulina platensis	PC	TLR2/ Myd88/NF-κB	PC inhibits the level of inflammatory factors in lung tissue, increases the relative expression of antioxidant enzymes, and down-regulates the activation of TLR2/ MyD88/NF-κB pathway in vivo.	[48, 49]
		Allergic airway inflammation		
Porphyra haitanensis	R-PC	T cells, mast cells	By blocking Th2 cell polarization and inhibiting antigen-stimulated release of allergic mediators in mast cells, R-PC reduces allergic sensitization to tropomyosin.	[18]
Bangia atropurpurea	R-PC	Dendritic Cells	R-PC promotes the transformation of dendritic cell immune function to Th1 activity.	[19]

2.5. Phycocyanin and brain diseases

Brain diseases such as stroke, Alzheimer's disease, ischemic brain injury etc., have always been a worldwide concern in human health. Brain diseases are considered due to the destruction of homeostasis in brain neural network, which in turn causes abnormal metabolism and apoptosis of neuronal cells. These courses are usually related to inflammatory damage. The antioxidant and anti-inflammatory effects of phycocyanin can lessen the oxidative stress response of neurons, reduce inflammation and regulate immunity, and then alleviate the damage

caused by brain diseases to a certain extent.

2.5.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurological disease coupled with severe cognitive impairment. At present, AD remains incurable, and brain inflammation and apoptosis are common symptoms of the disease. Drugs that prevent neuroinflammation and apoptosis will help the treatment of AD patients [50].

In diseases such as Alzheimer's or Parkinson's disease, fibrillary formation mediated by misfolding and aggregation of alpha-synuclein (αS) and amyloid -β(Aβ) is an important hallmark [51]. Liu et al. [52] found that PC could effectively inhibit the formation of A53TαS amyloid in a substoichiometric ratio (5:1). In addition, molecular interaction studies found that the mechanism of inhibiting fibril formation may be related to the unstable interaction between PC and αS.

Agrawal [53] induced AD in rats with 3 mg/kg streptozotocin (STZ), and studied the effect of PC on the improvement of cognitive dysfunction in AD models. The STZ induction led to an increase in cholinesterase and BAX activities, and a decrease in B-cell lymphoma-2 (BCL-2) and choline acetyltransferase (ChAT) activities. In addition, dysfunctional insulin signaling and reduced PI3K and AKT gene expression were also observed. The PC treatment significantly prevented the streptozocin (STZ)-induced increase in hippocampal cholinesterase and BCL2 associated X (BAX) activity and the increase in BCL-2 and ChAT levels. Neuroinflammation was significantly decreased, and the levels of brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) were up-regulated. As well, PC could alleviate dysfunctional insulin signals by increasing the expression of Insulin receptor substrate-1 (IRS-1), PI3K, and AKT genes, and finally play an inhibitory role for AD. (Table 1).

2.5.2. Ischemic brain injury

Cerebral infarction is an acute disease that affects the circulation of blood through brain, resulting in damage of nerve functioning. After cerebral infarction, many mechanisms would be involved in ischemic brain injury, including loss of cell homeostasis, elevated intracellular calcium levels, ROS-mediated toxicity, blood-brain barrier destruction, and leukocyte infiltration etc. [54]. The inflammatory response is an important part of ischemic injury.

Pavon-Fuentes et al.[55] studied the protection of PCB from endothelin-1 induced focal cerebral ischemia in rat. Through intraperitoneal injection of 50, 100, and 200 μg/kg PCB, the expression of brain myelin basic protein (MBP) and CNPase were detected in immunoelectron microscopy. The results show that PCB could improve the ischemic damage of PC12 cells. Compared with the model group, PCB intervention significantly reduced the cerebral infarct volume of ischemic rats, and retained the surviving cortical neurons. Furthermore, PCB restored the expression levels of MBP and CNPase in ischemic rats. It is worth noting that PCB can promote HO-1 expression, and HO-1 is a key enzyme responsible for bilirubin production in the heme catabolism pathway; it is beneficial to the phenotypic protection of endothelial cells. Therefore, PCB has the potential to protect the blood-brain barrier [33].

Marin-Prida [56] studied the protection of PCB against PC12 cell damage induced by H_2O_2 and glutamate. As being assessed by the MTT method, 5 μM or 10 μM PCB treatment could prevent H_2O_2 and glutamate-induced PC12 from cell damage, and regulate 190 genes (93 up-regulated, 97 down-regulated). Moreover, PCB regulated 19 genes, which was mainly related to the harmful pro-inflammatory environment. After PCB treatment, the elevated interferon (IFN)-γ, IL-6, and IL-17A in the bilateral common carotid arteries occlusion (BCCAo) group was significantly decreased, while the synthesis of genes Mal, nicotinamide adenine dinucleotide (NADH) dehydrogenase, Bcl-2a1, and Baiap2 increased. Furthermore, PCB down-regulated the levels of cytokines in the striatum and serum, induced the expression of vascular endothelial growth factor (VEGF), reduced the oxidative stress induced by BCCAo, and protected against ischemic brain injury.

2.6. Clinical study of phycocyanin

At present, the clinical application of phycocyanin in inflammation-related diseases is not abundant, and most of them are based on *Spirulina* and its extracts. There are a total of 4 clinical trials known so far, of which 3 are reported in articles, and the other one from a French company is only reported on the Internet.

In 2013, Jaideep Mahendra et al. [57] reported the clinical efficacy of *Spirulina* gel for the treatment of chronic periodontitis. The study selected 64 patients with a minimum probing pocket depth (PPD) $\geq$ 5 mm in the anterior mandible, treated with Scaling and Root Planning (SRP) and *Spirulina* gel, or SRP alone. The results show that patients treated with *Spirulina* gel addition significantly improved the PPD and clinical attachment levels after 120 days of treatment. The authors believed that this is related to the anti-inflammatory activity of C-PC in *Spirulina*. *Spirulina* could inhibit the release of various inflammatory factors activated by ROS and promote wound healing.

In 2015, Santosh Patil et al. [58] applied *Spirulina* to treat oral submucous fibrosis (OSMF). OSMF is thought to be associated with oral inflammation and is a precursor to a cancer. The study selected 42 patients with OSMF and divided them into two groups, treated with *Spirulina* (500 mg/day) and Aloe vera (5 mg/day). The results show that compared to Aloe vera, the patients treated with *Spirulina* had significant improvement in the degree of mouth opening, oral ulcers, erosions etc., showing an application prospect of *Spirulina* in the treatment of OSMF.

In 2019, *Spirulina* was used in adjuvant chemotherapy to improve the immune function of tumor patients. Ge et al. [59] increased the intake of *Spirulina* by 100 mg/time, 3 times/day, and take *Spirulina* for the first two cycles of conventional chemotherapy. After four rounds of chemotherapy treatments, IgM levels and $CD8^+$ T cell numbers increased in the *Spirulina*-treated group, while the control group showed a decrease. This suggests that *Spirulina* is able to alleviate myelosuppression after chemotherapy for malignant tumors and improve the immune function of patients.

In addition, a French company, AlgoSource, announced in 2022 that it will conduct a clinical trial of its PHYCOCARE® product for gastrointestinal cancer. PHYCOCARE® is a concentrated *Spirulina* bioactive liquid extract. The study will include 110 men and women in age of 18 years or older with gastrointestinal cancers, including esophageal, colorectal, or pancreatic cancers receiving oxaliplatin chemotherapy. So far, no relevant papers have been published in this clinical study.

3. Phycocyanin-excellent anti-inflammatory active substance

3.1. The direct anti-inflammatory pathway of phycocyanin

Direct anti-inflammatory effect refers to the inhibition of tissue inflammation through direct inflammatory pathways. The signal pathways related to phycocyanin anti-inflammatory include mainly the TLR and NF-κB pathways.

3.1.1. TLR pathway

TLR are a class of transmembrane protein receptor that can recognize and bind different molecules, activate its mediated signal transduction pathway, and induce the expression of immune factors. Like innate markers, TLRs expression is regarded as being restricted to immune cells. However, more and more evidence shows that the expression of TLRs is more diversified, and almost all cell lines are expressed, including epithelial cells, endothelial cells, nerve cells etc., which is essential to the tissue specificity of inflammation [70].

The activity of PC is thought to be related to the TLRs pathway. TLR2 mediates the secretion of cytokines that are proinflammatory through the p38 NF-κB and extracellular regulated protein kinases (ERK)-AP-1

pathways. Li et al.[48] found that PC can alleviate the inflammation in wild-type mice induced by bleomycin, but has no significant effect on the inflammation in $TLR^{-/-}$ mice, indicating that TLR2 pathway is involved in the anti-inflammatory function of PC. In addition, Liu et al. [49] discovered that PC pretreatment could block the TLR2/NF-κB pathway and reduce the levels of IL-6 and TNF-α.

The effect of PC on TLR4 is similar to that of TLR2, and it also reduces the expression of inflammatory factors by blocking the TLR4-mediated NF-κB pathway [26].

3.1.2. NF-κB pathway

The nuclear factor kappa-B (NF-κB) is a common protein complex. It is currently found that NF-κB can control a complex signal network through a long list of targets, and thus affect different cells. It is now known that various stimuli, including inflammatory factors, bacteria, or viruses can activate the NF-κB pathway, thereby mediate anti-apoptosis, cell adhesion, stress response, inflammation, and immune response [71]. At the same time, as a transcription factor located downstream of the TLR pathway, NF-κB can be mediated by the TLR pathway to regulate immune and inflammation-related factors [72]. Considering that the NF-κB pathway can also be directly activated by factors other than TLRs (such as IL-1, TNF-α etc.) [73], we will discuss the NF-κB pathway separately from the TLR pathway.

There are relatively many studies on phycocyanin inhibition of the NF-κB pathway in different disease models. A study on PC treatment of pulmonary fibrosis shows that PC can inhibit pulmonary fibrosis and reduce the level of tissue inflammation through the TGF-β/NF-κB pathway [49]. Alzokaky et al. [25] in a 2020 study presented that PC pretreatment could significantly reduce the expression of high mobility group protein (HMGB1) by inhibiting NOD-like receptor protein 3 (NLRP3)/NF-κB, oxidation markers, IL-1β, TNF-α, and ulcer index values, to protect gastric ulcers.

In addition to disease models, PC can act on immune cells, especially macrophages. Hao et al. [74] showed that PC could reduce the expression of inflammatory factors in LPS-induced macrophage by preventing the activity of NF-κB and reducing tissue inflammatory response. Zhu [31] studied Se-PC against inflammatory bowel disease and confirmed that Se-PC inhibited NF-κB nuclear translocation, reduced the transcription of pro-inflammatory cytokines, and inhibited the activation of macrophages and reduced the inflammatory bowel disease.

3.2. Indirect anti-inflammatory pathway of phycocyanin

The regulation of inflammatory response is a complicated issue. In addition to direct inflammation pathways, it is closely related to the pathways such as oxidation and apoptosis. Therefore, the indirect anti-inflammatory effect means to indirectly inhibit the development of inflammation by acting on signal pathways such as oxidation and apoptosis, thus reducing and restoring tissue inflammation.

3.2.1. Keap1-Nrf2 pathway

Oxidative damage is an important factor in inflammation development. The most commonly accumulated tissue inflammation is caused by oxidative damage. Therefore, oxidative pathways can indirectly affect the inflammatory response. It has been reported that LPS can induce macrophages to secrete TGF-β1, elevated TGF-β1 acts on TGF-β receptors, and further enhance the expression of ROS in cells [75]. Since the NF-κB pathway is activated by high levels of ROS in tissues, immune cells accelerate the secretion of inflammatory factors, which leads to increased inflammation in the tissues [76]. PC has a good antioxidant effect, and its anti-inflammatory activity is also believed to be related to its antioxidant effect.

The Keap1-Nrf2-ARE pathway is one of the important pathways that regulate the oxidative and inflammatory state of the body. As a redox-sensitive transcription factor, Nrf2 can regulate the expression of intracellular free radical scavengers. When oxidative stress occurs, Nrf2 is motivated, and accordingly the Keap1-Nrf2-ARE pathway is activated, the transcription and expression of downstream target genes nicotinamide adenine dinucleotide phosphate (NADPH), NAD(P)H:quinone oxidoreductase 1 (NQO1), GSH-Px etc., are regulated, and cells and tissues are protected from oxidative stress damage [77].

Liu et al. [28] studied X-ray-induced liver damage and showed that PC induction could activate the Nrf2 signaling pathway in mice to reduce X-ray-induced oxidative stress damage. Kim [78] in 2018 found that PC could mediate the synthesis of HO-1 through the protein kinase C (PKC) α/β II-Nrf2/HO-1 pathway and inhibit UV-induced apoptosis in primary skin cells. Methylglyoxal is an active dicarboxylic compound that commonly accumulates in diabetic patients and can have deleterious effects on the body. Gao et al. [79] found that PC could activate Nrf2 and the antioxidant enzyme HO-1 to prevent methylglyoxal-induced mitochondrial-dependent apoptosis in pancreatic α cells INS-1.

In addition, Okamoto et al. [80] fed mice with *Spirulina* supplemented diet and found that HO-1 and Nrf2 expressions increased in the mouse retina afterward, and the level of ROS decreased, which protected the mouse retina from light-induced damage. In a study on brain inflammation in neonatal rats, Patil et al. found that giving nursing mothers a *Spirulina*-rich diet could effectively reduce neuroinflammation in neonatal rats and increase the expression of Nrf2 in the cerebral cortex.

3.2.2. Apoptosis pathway

Apoptosis is an important process of programmed cell death that is essential for the body to eliminate unwanted cells and maintain normal physiological functions. Apoptosis is of great importance to the growth and development of organs and tissues, immunity, metabolism, and the removal of abnormal cells. As a protease that promotes apoptosis, caspase is a central part of the network of apoptotic mechanisms [81,82]. Among them, caspase-3 is the most critical executor of apoptosis, which can act on DNA-PK, PKC, poly ADP-ribose polymerase (PARP), and other substrates [83]. In the process of inflammation, tissue cell apoptosis occurs frequently. When expression of apoptosis signaling pathway is depressed, the tissue damage caused by inflammation can be alleviated.

Leung et al. [43] found that after intervention with PC, the pro-apoptotic protein caspase-3 was decreased and the anti-apoptotic protein Bcl-2 was increased in the lung, by which LPS-induced pulmonary inflammation was alleviated. Kim et al. [78] also showed that PC could protect UVB-induced cell apoptosis, reduce p53 and Bax expression, as well as inhibit caspase-3. Therefore, PC is capable of activating caspase signaling pathways, inhibiting caspase-3 activation, increasing the expression of antiapoptotic proteins Bcl-2 etc., and alleviating cell apoptosis caused by inflammation in the tissues.

4. Outlook

In a series of studies on the anti-inflammatory activity of phycocyanin, we found an interesting issue that phycocyanorubin (PCR), as a metabolite of phycocyanobilin, has a very similar structure to bilirubin [84]. The physiological activity of bilirubin has been verified in many studies, that is, bilirubin inhibits the binding of Keap1 and Nrf2 through the electrophilic addition of two propionate groups in its structure, so that Nrf2 can be translocated to the nucleus to promote the expression of downstream HO-1 [21], while the expressed HO-1 can act as antioxidant [85], anti-inflammatory [86,87] agent for regulating immune cell activity [20,88], and inhibit tissue inflammation. Therefore, we speculate that PCR may also has anti-inflammatory activity through Keap1-Nrf2-HO-1 pathway. In other words, the anti-inflammatory activity of phycocyanin is mainly caused by PCB.

When *Spirulina* or PC is taken orally into the human body as food or medicine supplement, it would be digested into amino acids and small peptides in the gastrointestinal tract. PCB encapsulated in PC is not affected by protease and exists in the form of PCB binding peptide

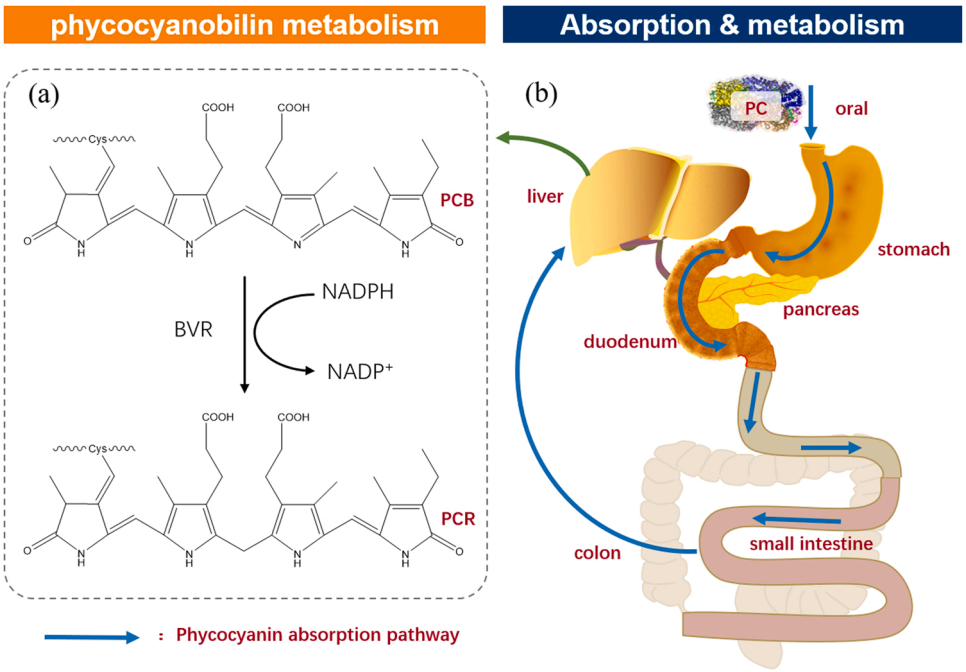

Fig. 1. Phycocyanin absorption metabolic pathway. (a): metabolism of phycocyanobilin in the liver; (b): the absorption and metabolism process of phycocyanin in the body.

because it is connected to the peptide segment through sulfide bond. Studies on mouse pulmonary fibrosis by Xie et al. [68] and Li et al. [69] showed that PC could regulate the composition of intestinal microorganisms in pulmonary fibrosis suffered mice and reduce mouse pulmonary fibrosis caused by bleomycin or radiation. In addition, in a study of alcoholic liver in mice [89], oral *Spirulina* could regulate intestinal microbiota, activate intestinal immune system, and participate in inhibiting liver inflammation in mice. This suggests that phycocyanin can change the composition of intestinal microorganisms after ingestion, and indirectly regulate the inflammation of lung and liver through intestine-lung axis and intestine-liver axis. Meanwhile, the digested small molecule PCB binding peptide is absorbed into blood through the small intestine. The results of Strasky et al. [33] show that PCB can simulate the products of heme catabolic pathway to protect arteriosclerosis, reduce the recruitment of immune cells, and reduce the symptoms of arteriosclerosis.

Previous studies have shown that PCB is metabolized in liver [84]. PCB could be recognized by biliverdin reductase (BVR) and reduced to a bilirubin structural analogue named phycocyanorubin (PCR) because of its linear tetrapyrrole structure similar to biliverdin (Fig. 1) [84]. PCR then are discharged into the small intestine together with other bile pigments through the bile duct, which acts as an anti-inflammatory in the small intestine and inhibits the development of colitis [31]. PCR is then absorbed into the blood through the villi of the small intestine, and transported to the lungs via systematic and pulmonary circulation, showing an anti-inflammatory effect and inhibition of pneumonia development [49], pulmonary fibrosis [48], and lung injury [43].

HO-1 is an essential enzyme responsible for removing heme that produced by aging blood cells. HO-1 converts heme into biliverdin by peroxidation, and then reduces it to bilirubin by biliverdin reductase, so as to remove excess heme in the body and maintain the internal redox balance of the body. Meanwhile, HO-1 has anti-inflammatory effects and can regulate various immune cells [20], which is related to the polarization of macrophages. The HO-1 can induce the transformation of macrophages from M1 to M2 type, and convert macrophages into anti-inflammatory type [88]. In the body, bilirubin formed by heme metabolism can in turn induce HO-1 expression, which is achieved through Keap1-Nrf2 pathway. Normally, Nrf2 and Keap1 in the cell are bound together, and Keap1 prevents the nuclear translocation of Nrf2. Joon et al. [21] demonstrated that bilirubin could produce electrophilic reaction through the Michael reaction acceptor (MRA) in its structure, bind to mercaptan group in Keap1, inhibit the binding of Keap1 and Nrf2, release Nrf2, transfer to the nucleus, and bind to ARE, as a promoter to the expression of downstream HO-1 gene. At the same time, the C10 double bond in bilirubin is reduced to a single bond, which can form an intramolecular hydrogen bond and enhance its electrophilic ability. Joon et al. [21] compared the Keap1 inhibition of biliverdin with bilirubin. By knocking out the BVR gene, they found that the induction of Nrf2 by biliverdin was significantly down-regulated after the lack of BVR. Therefore, biliverdin needs to be reduced to bilirubin in vivo to effectively induce the expression of Nrf2, although it contains electrophilic groups similar to bilirubin. The structure of PCR is similar to bilirubin, with two propionate groups and tetrapyrrole ring structure, and the double bond of C10 is also reduced to a single bond by BVR. Therefore, we speculate that the physiological activity of phycocyanin is mainly caused by PCB, and PCB is metabolized into PCR in vivo. PCR plays the same role as bilirubin in inducing the expression of HO-1 and reducing tissue inflammatory response.

Taking LPS induction as an example, we believe that LPS acts on the CD14 receptor to induce the expression of cell TGF-β1, and the overexpressed TGF-β1 acts on the TGF-β receptor on the cell, which increases the content of ROS in cells [75]; in addition, LPS can also act on TLR4 or TLR2. The elevated ROS and TLR4 combined with LPS can promote the expression of NF-κB in cells, and increase additionally the expressions of intracellular NO, IL-6, TNF-α, or other inflammatory factors, leading to the occurrence of intracellular inflammation [76]. The ingestion of PCR into cells can induce the synthesis of HO-1, and the elevated HO-1 can be mainly obtained by inhibiting the expression of NF-κB in different ways. First, because of its excellent antioxidant capacity, HO-1 can effectively reduce the expression of ROS in cells and directly block the subsequent tissue inflammation caused by ROS. Secondly, HO-1 can directly reduce the expression of NF-κB [86], and decrease the release of tissues inflammatory factors. In addition, HO-1 can induce IL-10 expression [20], and play an anti-inflammatory role in reducing the expression of NF-κB [90]. As mentioned above, HO-1 can regulate immune cells and induce phenotypic transformation of macrophages. The transformed M2

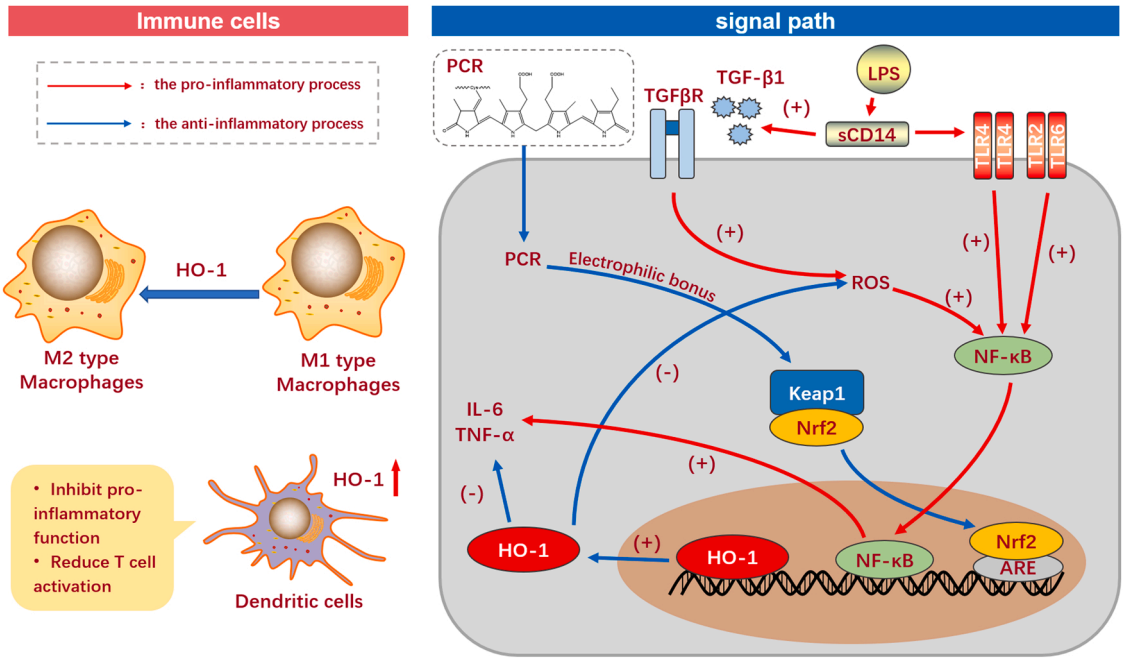

Fig. 2. The mechanism of phycocyanin anti-inflammatory activity [20].

macrophages can reduce phosphorylation of NF-κB by having an anti-inflammatory effect (Fig. 2) [88].

In addition, the bioavailability of PC is also a very interesting issue. As a macromolecular protein, PC could be decomposed into a complex mixture in gastrointestinal tract during oral administration, which affects the study of its availability. Therefore, it is necessary to propose possible small molecule active sites based on the structure of PC, and to study its bioavailability in a targeted manner. As mentioned above, PCB may become the focus of PC physiological activity research. PCB or PCB peptides have a definite structure. After entering the body via oral routes, they could not be degraded into complex mixtures, but specific products exist. Its presence can be easily detected in vivo. Therefore, subsequent research can focus more on the activity and bioavailability of PCB in vivo, and deepen the research and application of PC.

In this review, we summarized the anti-inflammatory effects and pathways of phycocyanin in different diseases, and proposed the mechanism of phycocyanin absorption, metabolism, and action in the body. In the theory we constructed, phycocyanin induces the expression of HO-1 through the PCB contained therein, and plays a variety of anti-inflammatory activities. In follow-up studies, more attention should be given to whether PC can increase the expression of HO-1 in the anti-inflammatory process of various organs and serum, and whether PCB can be found in both organs and serum. Research into the anti-inflammatory activity of phycocyanin will promote the application of phycocyanin in medical field for new anti-inflammatory drugs, and increase the market value of phycocyanin.

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Authors' contributions

Song Qin: Supervision. **Runze Liu:** Writing - Original Draft. **Wenjun Li:** Writing - Review & Editing.

Conflicts of interest statement

Runze Liu, Song Qin and Wenjun Li declare that they have no conflict of interest.

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