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Astaxanthin ameliorates cerulein-induced acute pancreatitis in mice

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ABSTRACT

Background: A various of pharmacological effects of astaxanthin has been confirmed. However, the mechanism underlying protective effect of astaxanthin on acute pancreatitis (AP) induced by cerulein still unclear. The present study is to investigate the mechanism underlying the effect of astaxanthin on autophagy and apoptosis via the JAK/STAT3 pathway.

Methods: Intraperitoneal injection of cerulein at hourly intervals followed by lipopolysaccharide injection were used in Balb/C mice. Vehicle or astaxanthin, which intraperitoneal injected in two doses (20 mg/kg and 40 mg/kg), were injected in mice 1 h before the first cerulein injection. At 3 h after the last injection, when the pathological changes were most severe, pancreatic tissue was analyzed by pathologically scored and hematoxylin and eosin (H&E) staining. The severity of AP was assessed by histological grading, proinflammatory cytokine levels, biochemistry, myeloperoxidase (MPO) activity, and analysis of JAK/STAT3 activity.

Results: Astaxanthin administration markedly reduced serum digestive enzyme activities, pancreatic histological scores, proinflammatory cytokine levels (tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6)), MPO and JAK/STAT3 activity.

Conclusion: Collectively, these results indicate that astaxanthin inhibits pancreatic injury in AP by targeting JAK/STAT3-mediated apoptosis and autophagy.

1. Introduction

Acute pancreatitis (AP), an acute inflammation of the pancreas, begins with intrapancreatic/acinar activation of trypsin and culminates in a series of varying degrees of severity, which include multiple organ failure and death. The incidence of AP is increasing globally, and mortality can be high among patients with organ failure and infected necrosis [1]. The activation of zymogens in acinar cells and release of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) are the initial events in acinar cells. Irrespective of the etiology, AP mounts an early systemic inflammatory response syndrome the magnitude of which determines the minimal to life-threatening early multiorgan dysfunction and mortality. Later in the clinical course (second week onwards), the infected necrosis in patients triggers a second wave of systemic inflammatory response syndrome and multiorgan dysfunction syndrome that is responsible for the second wave of mortality [2]. AP is initiated in two phases in general. Intracellular enzyme activation occurs firstly, resulting in acinar cell injury. Secondly, a pancreatic inflammatory response occurs [3]. Diverse proinflammatory cytokines play a crucial role in inflammation associated with AP [4]. Although extensive research direct at elucidating the pathogenesis of AP, the specific mechanism remains needs to be elucidated.

Screen for appropriate drugs for pancreatitis depend on the establishment of animal models which must closely mimicking the pathological process in the body. At present, the main methods to establish an AP model are intraperitoneal injection of cerulein, intraperitoneal injection of L-arginine, retrograde injection of sodium taurocholate, choline - deficient ethionine diets, and pancreatic duct ligation. Cerulein is an analogue of cholecystokinin, which can stimulate the secretion of gastrointestinal hormones. The mechanism by which cerulein induces AP is through the cholecystokinin receptor, which stimulates the pancreatic acinar cells and activates lysosomal enzymes to hydrolyze tissue protein B, leading to trypsinogen translated into trypsin and “self-digestion” of the pancreas [5]. The merits of the

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Table 1
The criteria of pancreatic histological score.

	Histological score	Pathologic change
Inflammation	0	None
	1	Inflammatory cells present at interlobular areas
	2	Present at intralobular areas
	3	Present at interacini
Cell necrosis	0	None
	1	< 10% necrosis
	2	< 40% necrosis
	3	> 40%necrosis
Vacuolization	0	None
	1	< 20% acini with vacuoles
	2	< 50% acini
	3	> 50% acini
Acinar edema	0	None
	1	Interlobular edema
	2	Intralobular edema
	3	Interacinar edema

Table 2
Primer sequences for qRT-PCR.

Gene	Primer sequence (5′-3′)
IL-1β	Forward: CGATCGCGCAGGGGCTGGGCGG
	Reverse: AGGAACTGACGGTACTGATGGA
IL-6	Forward: CTGCAAGAGACTTCCATCCAG
	Reverse: AGTGGTATAGACAGGTCTGTTGG
TNF-α	Forward: CAGGCGGTGCCTATGTCTC
	Reverse: CGATCACCCCGAAGTTCAGTAG
Bcl-2	Forward: GCTACCGTCGTCGTGACTTCGC
	Reverse: CCCCACCGAACTCAAAGAAGG
Bax	Forward: AGACAGGGGCCTTTTTGCTAC
	Reverse: AATTCGCCGGAGACACTCG
LC3	Forward: GACCGCTGTAAGGAGGTGC
	Reverse: AGAAGCCGAAGGTTTCTTGGG
Beclin-1	Forward: ATGGAGGGGTCTAAGGCGTC
	Reverse: TGGGCTGTGGTAAGTAATGGA
P62	Forward: GAGGCACCCCGAAACATGG
	Reverse: ACTTATAGCGAGTTCCCACCA
β-Actin	Forward: GGCTGTATTCCCCTCCATCG
	Reverse: CCAGTTGGTAACAATGCCATGT
Caspase-3	Forward: CTCGCTCTGGTACGGATGTG
	Reverse: TCCCATAAATGACCCCTTCATCA
Caspase-9	Forward: GGCTGTTAAACCCCTAGACCA
	Reverse: TGACGGGTCCAGCTTCACTA
JAK1	Forward: AGTGCAGTATCTCTCCTCTCTG
	Reverse: GATTCGGTTCGGAGCGTACC
JAK2	Forward: GGAATGGCCTGCCTTACAATG
	Reverse: TGGCTCTATCTGCTTCACAGAA T

cerulein model include rapid induction, noninvasiveness, good reproducibility, strong applicability, and the similarity to clinical process and disease, and it is the most commonly used model.

Astaxanthin is a xanthophyll carotenoid found in various microorganisms and marine animals. It contains conjugated double bonds, hydroxyl and keto moieties, not only has the medicinal properties such as diabetes and cardiovascular disease [6, 7], but also anti-inflammatory effects and anti-oxidative stress effects [8–10]. Astaxanthin consists of two asymmetric carbons located at the 3, 3′ positions of the β-ionone ring with hydroxyl group (−OH) on either end of the molecule [11, 12]. The type of conjugated double bond acts as a strong antioxidant by donating the electrons and reacting with free radicals to convert them to be more stable product and terminate free radical chain reaction [8]. Astaxanthin is a potent antioxidant to terminate the induction of inflammation. Such as astaxanthin significantly reduced gastric inflammation in H. pylori-infected mice [13, 14]; and reduced the DNA oxidative damage biomarker inflammation [15]. It is reported that astaxanthin is a promising molecule for the treatment of ocular inflammation in eyes [16, 17]. The Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway plays a fundamental role in the inflammatory response [18]. STAT3 is recognized as the intersection of multiple signal transduction pathways. JAK/STAT3 is the classic pathway of STAT3 signal transduction and transcriptional activation. A variety of drugs produce therapeutic effect by regulating the JAK/STAT3 signal transduction pathway [19–21]. However, the role of the JAK/STAT3 signaling pathway on apoptosis in AP remains unclear.

Previous studies showed that the pathogenesis of AP caused by cerulein involved apoptosis and autophagy [22–25]. Apoptosis is triggered by extrinsic and intrinsic pathways [26]. Autophagy, manifest as the formation of autophagosomes and autolysosomes leading to the massive degradation of organelles, is a catabolic process. IL-6, is an important cytokine in stress responses that is regulated by inflammatory factors such as TNF-α and IL-1; it can induce acute phase protein synthesis, aggravate and amplify inflammatory reactions, and damage tissue [27]. During the development of AP, TNF-α can initiate inflammatory responses [28], in which the mechanism is the induction of a large number of inflammatory factors, such as IL-1 and IL-6, which can also lead to TNF-α upregulation in turn. The “TNF-α- inflammatory factors - TNF-α” inflammatory response can damage to endothelial cells, increase vascular permeability, cause local microcirculatory disorders, and further aggravate the injury to the pancreas.

In present study, the effect of astaxanthin on cerulein-induced AP we investigated in a mice model. The levels of digestive enzyme and pro-inflammatory mediators including TNF-α, IL-1β, and IL-6, myeloperoxidase (MPO) activity and pancreatic histological changes were investigated to gain insight into the protective effects of astaxanthin.

2. Materials and methods

2.1. Reagents

Astaxanthin, cerulein, and lipopolysaccharide were purchased from Sigma–Aldrich (St. Louis, MO, USA), and the antibodies were from Cell Signaling Technology (Danvers, MA, USA), including antibodies against JAK1, JAK2, STAT3, P-STAT3, p62, LC3, Beclin1, IL-6, TNF-α, IL-1β, Bcl-2, Bax, Caspase3 and Caspase9. The amylase and lipase enzyme linked immunosorbent assay (ELISA) kits were purchased from Shanghai Westang Bio-tech Co. Ltd. (Westang Bio-tech, shanghai, China). The RNA polymerase chain reaction (PCR) kit was purchased from Takara (TaKaRa Biotechnology, Dalian, China). IL-6, TNF-α, and IL-1β ELISA kits were purchased from Anogen-Yes Biotech Laboratories Ltd. (Anogen-Yes Biotech, Mississauga, Canada). Myeloperoxidase assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Jiancheng Biotech, Nanjing, China).

2.2. Animals

Male Balb/c mice (6–8 weeks old, 18 ± 2 g) were purchased from Shanghai Laboratory Animal Co. Ltd. (SLAC, Shanghai, China). The mice were housed in a clean room with controlled light-dark cycles (12 h: 12-hour light: dark) and a controlled-temperature (23 ± 2 °C) and humidity of 50%, with free access to food and water. All animal experiments were approved by the Animal Care and Use Committee of Shanghai Tongji University, China and were conscientiously implemented according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.3. Preliminary study

A total of 48 mice were randomly divided into four groups as follows: a normal control (NC) group treated with saline solution, an olive oil group, and two astaxanthin groups treated with astaxanthin at doses of 20 mg/kg and 40 mg/kg. Astaxanthin was dissolved in oil. Five mice