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Suppression of inducible nitric oxide synthase and tumor necrosis factor-alpha level by lycopene is comparable to methylprednisolone in acute pancreatitis

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ABSTRACT

Background: Oxidative stress and inflammation may play a key role in the pathogenesis of acute pancreatitis (AP). Lycopene, a natural carotenoid, has antioxidant scavenger capacity and inhibits inflammation in many experimental models.
Aim: The study was designed to investigate whether lycopene can ameliorate L-arginine-induced pancreatitis in rats and to elucidate the underlying molecular mechanisms of these effects.
Methods: Forty-eight adult male Wistar rats were divided into: control group (vehicle, orally, 10 days), AP group (3 g/kg L-arginine, single i.p. injection, on day 10th of the experiment), lycopene group (50 mg/kg) and methylprednisolone group (30 mg/kg). Lycopene and methylprednisolone were given orally, once daily for 10 days prior to L-arginine injection. Rats were sacrificed 24 h after L-arginine injection. Inflammation/oxidative stress and pancreatic markers were assessed. Pancreatic histopathological studies were done.
Results: Lycopene group showed a significant reduction in tumor necrosis factor alpha (TNF-α), myeloperoxidase activity, and down-regulation of inducible nitric oxide synthase (iNOS) gene expression. Pancreatic nitric oxide concentration was reduced and pancreatic GSH was increased in lycopene group. Serum α-amylase and lipase activities were reduced by lycopene treatment. The histology of pancreas was improved in lycopene group as well as methylprednisolone group.
Conclusion: Lycopene prior treatment proved anti-inflammatory and antioxidant effects against AP rat model *via* different mechanisms.

1. Introduction

Complementary and alternative medicine has been widely integrated in the treatment of various diseases including gastrointestinal and liver disorders through using natural materials based on clinical and research experiments [1]. Hence, there is a need for potential antioxidant and anti-inflammatory agents available from natural sources that have fewer side effects to control the underlying pathogenesis of acute pancreatitis (AP) [2].

Lycopene (LYC) is a non-provitamin A carotenoid, found in red colored fruits and vegetables such as tomato, papaya, pink grapefruit, and watermelon [3]. A high number of conjugated double bonds present in LYC makes it a very powerful antioxidant compared to β-carotene or α-tocopherol. LYC acts as a scavenger of several free radical species and preventing the oxidative damage of critical biomolecules including lipids, proteins, and DNA [4,5]. Furthermore, LYC has anti-angiogenic, anticancer, antioxidant, and anti-inflammatory properties which have been established both *in vitro* and *in vivo* [6,7]. LYC can inhibit nuclear factor kappa B (NF-κB) activation and interleukin-6 (IL-6) expression in pancreatic acinar cells and reduce peroxynitrite and oxidative stress involved in inflammatory diseases [8].

AP is currently one of the most common reasons for hospitalization with a gastrointestinal condition. Approximately 80% of patients admitted with AP have mild, self-limited disease and the overall mortality is approximately 2% [9]. The most common causes of AP are gallstones and alcohol abuse. Moreover, many other etiologies are documented such as; drugs (tetracycline, estrogens, and furosemide), viral infection (mumps and hepatitis), hypercal-

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cemia, hypertriglyceridemia, and developmental abnormalities of the pancreas [10].

The pathogenesis of AP is complicated and poorly understood. However, oxidative and nitrosative stresses, inflammation, and tissue necrosis may play a significant role in the pathogenesis [11]. Upregulation of inflammatory mediators including interleukin-1β (IL-1β), IL-6, tumor necrosis factor (TNF-α), nitric oxide (NO), and production of adhesion molecules are markedly observed during AP. These mediators initiate and amplify a cascade of cytokines, leading to the development of AP from a local to a systemically complicated disease. The cumulative effect of these mediators eventually leads to vascular leakage, hypovolemia, systemic inflammatory response syndrome, shock, and organ failure [12].

L-Arginine (Arg) is an essential amino acid that has been used to induce severe necrotizing AP in rats without any morphological changes in islets of Langerhans [13]. The mechanism by which Arg causes AP may be through releasing oxygen-free radicals, NO, and inflammatory mediators that have a key role in the development of the disease [14].

Therefore, the present study was conducted to elucidate the potential alleviating effect of LYC against Arg-induced AP in male rats and to investigate the molecular mechanisms underlying these effects. The potential effects of LYC were compared with those obtained by methylprednisolone (MP). MP is a widely used anti-inflammatory and immunosuppressive therapy in both acute and chronic inflammatory diseases [15].

2. Materials and methods

2.1. Study design & treatments

The study was conducted in accordance with guidelines for care and use of laboratory animals approved by Research Ethics Committee of Faculty of Pharmacy, Tanta University (Egypt). Forty-eight adult male Wistar albino rats weighing (170–200 g) were obtained from the National Research Center (Giza, Egypt). Rats were housed in wire cages, under constant environmental conditions for adaptation and allowed free access to tap water and standard rodent chow. After two weeks of acclimatization, rats were fasted for 12 h prior to receive drugs, and divided randomly into four equal groups (n = 12/group) for treatments as follows: control group: rats were given vehicle *via* oral gavage for 10 consecutive days. AP group: rats received single i.p. injection of 3 g/kg Arg (≥98% purity, Sigma–Aldrich, USA) on the 10th day of the experiment. Arg was prepared by dissolving Arg in normal saline to a final concentration of 300 mg/mL and the pH was adjusted to 7 [13]. LYC group: rats were treated with 50 mg/kg of LYC (≥90% purity, Changsha Staherb Natural Ingredients, China) *via* oral gavage for 10 consecutive days prior to Arg i.p. injection. LYC was prepared by dissolving LYC in pure corn oil (Iso Chem Co., Egypt) to a final concentration of 10 mg/mL. The dose of LYC was previously shown as an effective anti-inflammatory and antioxidant dose [16,17]. MP group: rats were treated with 30 mg/kg of MP (≥98% purity, Sigma Pharmaceutical Industries, Quweisna, Egypt) *via* oral gavage for 10 consecutive days prior to Arg i.p. injection. MP was prepared by dissolving MP in 3% Tween 80 (El-Nasr Pharmaceutical Co., Egypt) and normal saline to a final concentration of 6 mg/mL [2].

2.2. Specimen collection and analysis

Twenty-four hours after Arg injection, rats were weighed and anesthetized with light diethyl ether (EL-Nasr Pharmaceuticals Co., Egypt). Blood samples were collected *via* intracardiac puncture. Serum was separated at 800 × *g* at 4 °C for 15 min and stored at −20 °C for biochemical analysis of α-amylase and lipase activities. Rats were sacrificed and pancreas was dissected rapidly, trimmed of fats on ice, and weighed for determination of pancreatic edema [pancreas weight (mg)/body weight (g)] [18]. The pancreas was divided into 2 parts; one part was stored at −80 °C for assessment of inducible nitric oxide synthase (iNOS) gene expression and biochemical analysis of reduced glutathione (GSH), nitric oxide content (NO), tumor necrosis factor-α (TNF-α), myeloperoxidase (MPO) activity. The other part was fixed in 10% formalin for histological examination.

2.2.1. Histopathological examination

Pancreatic tissue sections were embedded in paraffin. 5 μm sections were processed using a microtome (Leica RM2155, Germany). Sections were stained with hematoxylin and eosin (H&E) and examined by expert pathologists using a light microscope (Leica DM 500, Switzerland). The histopathological changes such as pancreatic acinar cell necrosis, edema, blood vessels congestion, and leukocytes infiltration were assessed and scored as described by Schmidt's standards [19].

2.2.2. Determination of serum α-amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) activities

Serum amylase [20] and lipase [21] activities were determined by colorimetric methods using the commercially available kits (Reactivos GPL, Barcelona, Spain) for amylase and (Quimica Clinica Aplicada S.A, Spain) for lipase. Enzyme activities were expressed as U/L.

2.2.3. Determination of pancreatic reduced glutathione (GSH) concentration

Pancreatic GSH was measured colorimetrically according to Ellman [22] using Biodiagnostics kit (Giza, Egypt). Reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) by GSH yields a yellow colored product which was measured at 405 nm using Spectrophotometer (Evolution 300, Thermo Fisher Scientific, USA). GSH was expressed as mg/g tissue.

2.2.4. Determination of pancreatic nitric oxide (NO) concentration

Nitric oxide content of pancreatic tissue was measured as nitrite using Griess reagent which was prepared by mixing *N*-(1-naphthyl)-ethylenediamine (0.1%) and sulfanilamide (2%) in a ratio of 1:1 v/v [23]. The absorbance was measured at 540 nm. Sodium nitrite was used for preparing the standard curve. NO concentration was expressed as mM/g protein. Total protein concentration was determined according to the method described by Lowry et al. [24].

2.2.5. Determination of pancreatic tumor necrosis factor alpha (TNF-α) concentration

Pancreatic TNF-α concentration was measured by ELISA [25] using a kit purchased from Sunred Biological Technology (Shanghai, China) according to manufacturer protocol. TNF-α concentration was expressed as pg/g protein.

2.2.6. Determination of pancreatic myeloperoxidase (MPO, EC 1.11.2.2) activity

Pancreatic myeloperoxidase activity was measured according to Bradley et al. [26] using *O*-dianisidine as substrate. 50 mg of pancreatic tissue was homogenized in 50 mM of potassium phosphate buffer (pH 6) containing 0.5% hexadecyltrimethylammonium bromide (Oxford Laboratory Reagent Co., India). Samples were centrifuged at 12,000 × *g* for 15 min at 4 °C. 50 μL of supernatant was added to the reaction mixture (0.167 mg/mL of *O*-dianisidine (Sigma–Aldrich, USA) and 0.3% H_2O_2 solution) which was prepared

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