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Crocetin ester improves myocardial ischemia via Rho/ROCK/NF-κB pathway

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ABSTRACT

Crocetin ester (CE) is the active ingredient of *Crocus sativus* L. stigmas and *Gardenia jasminoides* Ellis fruit. The main purpose of the present study was to investigate the protective effect of CE on isoproterenol (ISO)-induced acute myocardial ischemia (AMI) through Rho/ROCK/NF-κB pathway and explore its underlying mechanism. Administration of CE (25 and 50 mg/kg) could significantly reduce the serum contents of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). In addition, pretreatment with CE attenuated the contents of creatine kinase (CK), malondialdehyde (MDA) and the activities of lactate dehydrogenase (LDH), superoxide dismutase (SOD) in serum. Treatment with CE also improved the histopathological alteration and decreased the ST elevation. Furthermore, CE could ameliorate the cardiac expressions of Cu, Zn-superoxide dismutase (SOD1), MDA5, Rho, ROCK, p-IκB and p-NF-κBp65 in ISO-induced rats. It was assumed that CE might be a new therapeutic candidate for the treatment of AMI possibly through the inhibition of Rho/ROCK/NF-κB pathway.

1. Introduction

Acute myocardial ischemia, caused by an imbalance between blood supply to the heart and demand of the myocardium, is one of the most serious coronary heart diseases and leads to relatively higher morbidity and mortality in the Western world [1]. In accordance with the World Health Organization, it will be the leading cause of death around the world [2]. The primary features of myocardial ischemia are hypoxia, ischemia and inflammation. Obstruction of blood flow to the heart contributes to the ischemia of myocardial cells which may contribute to apoptotic process [3]. Thus, it is critical to clarify the exact pathogenesis progression of AMI and investigate the potential mechanism by which CE protects heart from myocardial cell swelling, degeneration, transverse striation loss and inflammatory cell infiltration. Although significant progresses in basic research and clinical experiments have been made, there were limited fundamental breakthroughs in drug therapy. Currently, the pharmaceutical development of effective components from natural medicines is increasingly important.

Nature products are widely applied for the interventions of a variety of diseases [4,5]. Crocetin ester (CE), extracted from *Crocus sativus* L. stigmas and *Gardenia jasminoides* Ellis fruit, is the esterification product of crocetin ($C_{44}H_{64}O_{24}$) with one or two glucose, gentibiose or neapolitanose sugar moieties [6]. Pharmacological effects of CE are rarely reported in literatures. However, as a traditional Chinese medicine, saffron, especially the extract crocin, has been widely used to treat myocardial ischemia. Since CE is similar with crocin in many aspects [7], it is speculated that CE could also attenuate cardiac ischemia and hypoxia.

As a serine/threonine kinase, Rho-kinase has been identified as one of the downstream effector proteins of Rho. The Rho/ROCK signaling pathway is involved in pro-inflammatory molecule generations [8], cell adhesion, contraction of vascular smooth muscle cells, gene transcription [9] and endothelial NO production [10]. Previous studies also indicated that Rho/ROCK signaling played important roles in cardiovascular diseases [11]. As a classical anti-inflammatory pathway, NF-κB was

Abbreviations: AMI, acute myocardial ischemia; CE, crocetin ester; GEFs, guanine nucleotide exchanging factors; GAP, GTPase activating protein; GDI, GDP dissociation inhibitor; Pro, propranolol; ISO, isoproterenol.

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extensively reported to participate in ischemia and reperfusion [12,13]. Rho protein is also implicated in the regulation of NF-κB signal transduction cascades related to inflammation [14]. The relationship between Rho/ROCK and NF-κB signaling in myocardial ischemia has not been elucidated. Therefore, we carried out the experiments to verify our hypothesis that CE could protect rats from ISO-induced AMI through Rho/ROCK/NF-κB signaling pathway.

2. Materials and methods

2.1. Main reagents and kits

Crocetin ester (purity 99%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Propranolol (Pro) was supplied by Simcare Drug Store (Nanjing, China). TNF-α, IL-6 and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were provided by Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China). CK, LDH and ELISA kits for the detections of MDA and SOD were produced by Jiancheng Bioengineering Institute (Nanjing, China). Isoproterenol (ISO) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

50 Sprague–Dawley (SD) rats (180–220 g, 8 weeks old), provided by Comparative Medicine Centre of Yangzhou University, were housed in a specific pathogen-free (SPF) laboratory in the Animal Center of Nanjing University of Chinese Medicine under a 12 h light/12 h dark cycle circumstance at 22–24 °C with humidity of 40–70% and were access to food pellets and water ad libitum.

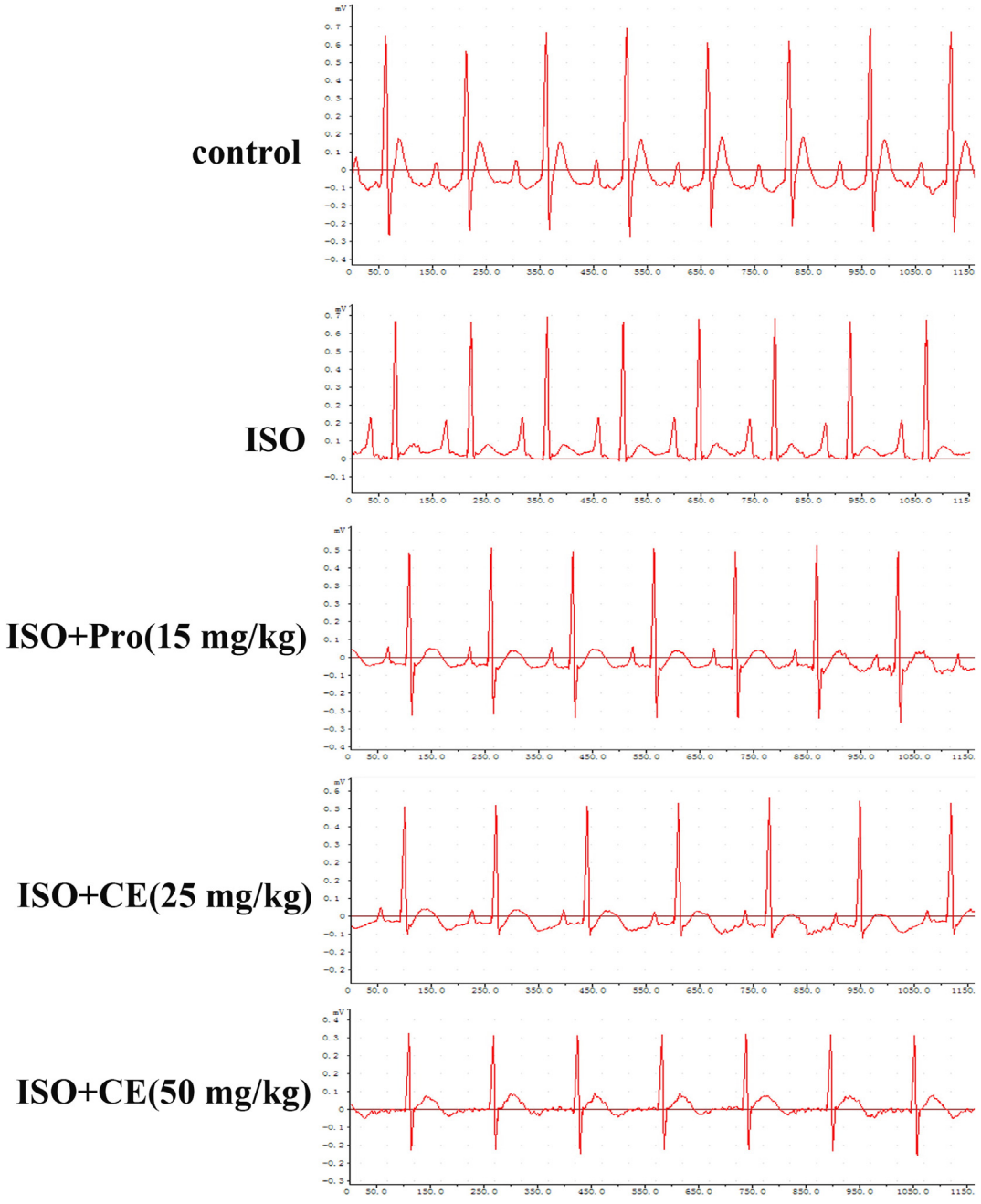

Fig. 1. Effect of CE on ST-segment elevation.