Saffron (Crocus sativus) intake provides nutritional preconditioning against myocardial ischemia—reperfusion injury in Wild Type and ApoE(−/−) mice: Involvement of Nrf2 activation

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KEYWORDS
Saffron; Myocardial infarct size; ApoE(−/−) mice; Molecular signaling; Nrf2

Abstract Background and aims: Saffron is an antioxidant herbal derivative; however, its efficacy as a nutritional cardioprotective agent has not been fully elucidated. We investigated the cardioprotective properties of a standardized saffron aqueous extract (SFE) against ischemia/reperfusion (I/R) injury in Wild-Type (WT) and ApoE(−/−) mice and the underlying molecular mechanisms.

Methods and results: WT and ApoE(−/−) mice were subjected to 30 min I and 2 h R, with the following per os interventions for 4 weeks: 1) WT Control Group, receiving Water for Injection (WFI); 2) WT Crocus Group, receiving SFE at a dose of 60 mg/kg/day; 3) WT Crocus + Wort group, receiving SFE as described above and wortmannin at a dose of 60 μg/kg bolus 15 min before R; 4) ApoE(−/−) Control Group, receiving WFI; 5) ApoE(−/−) Crocus Group, receiving SFE at a dose of 60 mg/kg/day and 6) ApoE(−/−) Crocus + Wort: receiving SFE as described above and wortmannin at a dose of 60 μg/kg bolus, 15 min before R. Ischemic area/area at risk (I/R%) ratio was measured. Blood samples and ischemic myocardial tissue were collected at the 10th min of reperfusion for assessment of troponin I, malondialdehyde (MDA), nitrotyrosine (NT), p-eNOS, eNOS, p-Akt, Akt, p-p42/p-p44, p-GSK3, IL-6, Nrf2, HO-1 and MnSOD expression. The effect of SFE on Nrf2 expression was also evaluated in vitro. SFE reduced infarct size in WT (16.15 ± 3.7% vs 41.57 ± 2.48%, ***p < 0.001) and in ApoE(−/−) mice (16.14 ± 1.47% vs 45.57 ± 1.73%, ***p < 0.001). The administration of wortmannin resulted in partial inhibition of the infarct size limitation efficacy of SFE (in both WT and ApoE(−/−) mice). Mice receiving SFE showed increased levels of eNOS, p-Akt, p-ERK1/2, p-44/p-42 and p-GSK3β-Ser9 and reduced expression of IL-6 and iNOS; furthermore, SFE reduced the levels of MDA and NT. SFE induced Nrf2 expression and its downstream targets, HO-1 and MnSOD in the myocardium of the treated animals, and induced Nrf2 expression in vitro in a dose-dependent manner.
Introduction

Modern dietary habits and lifestyle changes contribute to the prevention of worldwide epidemic diseases such as metabolic syndrome and hypercholesterolemia. The aforementioned morbidities are highly associated with increased prevalence of atherosclerosis-related cardiovascular diseases (CVDs) such as coronary artery disease, peripheral arterial disease and heart failure, which are the leading causes of morbidity and mortality [1]. Nutraceuticals or functional foods include any food or food constituents that can bestow beneficial effects on health status beyond their traditional nutritional use [2]. Oxidative stress is associated with the development of acute myocardial infarction (AMI) and atherosclerosis, antioxidant nutraceuticals can reduce the levels of reactive oxygen species (ROS), decelerating their progression and incidence and increasing life span [2].

Repeated and short sublethal ischemic preconditioning exerts protection of the myocardium against subsequent I/R. However, the clinical implication of ischemic preconditioning is limited because of ethical and practical reasons [4]. Pharmacological preconditioning represents an ideal alternative that may substitute the short ischemic insults for pharmacological means. Recently, nutritional preconditioning is considered a form of pharmacological preconditioning mediated by the intake of nutraceuticals through diet [5].

Saffron, the dried stigma originating from the flower of Crocus sativus L., has been used as a spice and dye pigment since antiquity [6]. Based on saffron traditional use, previous research has shown that saffron and its bioactive constituents exert anti-cancer [7], anti-oxidant, anti-inflammatory [8,9], lipid-lowering, insulin sensitization [10,11], and anti-depressant [12] actions. Saffron and especially its active constituents have shown protective effects on the myocardium against I/R [13,14] via protein kinase B (Akt)/endothelial nitric oxide synthase (eNOS)/glycogen synthase kinase 3 beta (GSK3β) axis [15], against isoproterenol-induced cardiotoxicity [16–18] and, also against norepinephrine induced myocardial hypertrophy [19].

Although saffron cardioprotective action has been investigated, current scientific approaches are mostly based on ex vivo models and do not take under consideration the prevalence of co-morbidities in the ischemic myocardium, where nutraceuticals can be used as promising pharmacological agents. Therefore, the aim of our study is to investigate whether saffron aqueous extract (SFE) could induce nutritional preconditioning, by means of infarct size limitation in vivo, in healthy myocardium and under endothelial dysfunction. To facilitate the latter purpose we have used a well established in vivo model of atherosclerosis and endothelial dysfunction, the ApoE(-/-) mice [20]. Subsequently, we sought to elucidate the underlying mechanisms of the protection in normal and ApoE(-/-) mice, focused on the molecular signaling of preconditioning.

Methods

For complete Materials and methods, see Supplementary material.

Experimental protocol

First series of experiments

24 male 20-week old C57BL/6j and 19 male 20-weeks old ApoE(-/-) mice receiving normal diet were used. These mice were randomly separated into six groups: 1) WT Control Group (n = 10), receiving Water for Injection (WFI) solution per os through gavage for 4 weeks; 2) WT Crocus Group (n = 8), receiving SFE per os diluted in 100 µL of WFI through gavage at a daily dose of 60 mg/kg, for 4 weeks; 3) WT Crocus + Wort group (n = 6), receiving SFE as described above and wortmannin at a dose of 60 µg/kg bolus 15 min before reperfusion [21,22]. Dose selection was based on a preliminary pharmacokinetic study where it was observed that a 60 mg/kg/day of SFE administered orally through gavage to the C57BL/6j wild type mice, led to measurable animal exposure to crocin, the main active ingredient of the administered SFE (data not shown), as well as on literature data [23]. 4) ApoE(-/-) Control Group (n = 7): receiving Water for Injection (WFI) solution per os through gavage for 4 weeks; 5) ApoE(-/-) Crocus Group (n = 6), receiving SFE per os diluted in 100 µL of WFI through gavage at a daily dose of 60 mg/kg, for 4 weeks and 6) ApoE(-/-) Wort Group (n = 6), receiving SFE as described above and wortmannin at a dose of 60 µg/kg 15 min bolus, before reperfusion [21,22]. Experimental protocol is illustrated in Fig. S8.

Second series of experiments

The aforementioned interventions were repeated up to the 10th minute of reperfusion, in order to obtain tissue samples from the ischemic area of the heart for Western Blot analysis and blood samples obtained via cardiac puncture for the assessment of malondialdehyde (MDA) levels as a biomarker for lipid peroxidation (WT Control...
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