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A chlorogenic acid-phospholipid complex ameliorates post-myocardial infarction inflammatory response mediated by mitochondrial reactive oxygen species in SAMP8 mice

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ABSTRACT

Mitochondrial reactive oxygen species (mtROS) directly stimulate the inflammatory cytokines cascades and participate in age-related changes of cardiovascular diseases. Application of small molecule targeting the mtROS is significant towards development of better therapy to combat inflammatory response after myocardial infarction (MI) in the aging heart. Chlorogenic acid (CGA) is a well-known natural compound while the clinical potential is largely stifled by its poor oral absorption. In the present study, we tested the protective effect of a novel chlorogenic acid-phospholipid complex (CGA-PC) against acute post-MI inflammation in aged senescence accelerated mouse model. 10-month-old SAMP8 mice were treated with CGA-PC (equivalent of CGA 10 or 20 mg/kg body weight) or phospholipid randomly by gavage on a daily basis for 2 weeks. mtROS, lipid peroxidation, H_2O_2 production and oxygen consumption were evaluated in hearts subjected to ischemia reperfusion (I/R) induced by left anterior descending artery ligation. CGA-PC significantly reduced pro-inflammatory cytokines and myocardial necrosis, accompanied by decreased oxidative stress and mitochondrial respiratory deficits. p-JNK, MnSOD and soluble cytochrome c were up-regulated in the necrotic heart tissue, while CGA-PC treatment increased the expression of MKP-1 and inhibited the downstream activation of JNK. Our study indicated that CGA-PC ameliorated post-MI inflammatory response in aging heart and that it might be a promising candidate for the clinical development of CGA.

1. Introduction

Clinical and experimental evidences show that myocardial infarction triggers an intense inflammatory response associated with essential cardiac repair and pathogenesis of heart failure. Turillazzi evaluated detectable morphological changes in myocardial specimens of fatal myocardial infarction (MI) patients and suggested essential markers (*i.e.*, IL-15 and MCP-1) as early indicators of inflammatory response to MI [1]. Oxidative stress, lipid peroxidation and inflammatory mediators or cytokines are considered as potential targets for therapeutic intervention. Several recent studies have revealed the approach to inhibit excessive inflammatory response to changes in myocardial structure and function, assigning to limiting reactive oxygen species (ROS) to being an epiphenomenon of the cardiac aging mechanism. However, ROS generated from multiple sources in the mammalian cell including xanthine oxidase, cytochrome p450 and mitochondrial electron transport chain, is both the major contributor and consequence of cardiac inflammation. Translation of ROS-scavenging treatment into therapy for patient with MI was unsuccessful and several classical anti-inflammatory approaches also failed to improve heart function in clinical investigation of

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heart failure. Recently, Age-related mitochondrial reactive oxygen species (mtROS) increase is discovered to contribute to inflammatory cytokine cascade *via* different mitogenactivated protein kinases (MAPK) mechanisms and, furthermore MAPK phosphatase-1 (MKP-1) plays a critical role in the regulation of cytokine expression upon myocardial infarction. While MKP-1 is the key negative regulator of JNK MAPK, little is known about the role of MKP-1 during post-myocardial infarction inflammatory response.

Chlorogenic acid (CGA), an ester of quinic acid and caffeic acid, is one of the most abundant phenolics in plants [2]. CGA distributed widely in coffee beans, strawberries, pineapples, apples, sunflowers, and blueberries. At the same time, CGA is also major active ingredient in many traditional Chinese medicines such as *Flos Lonicerae Japonicae* [3], *Flos Chrysanthemi Indici* [4], *Eucommia cortex* [5] and *Solanum lyratum* [6]. Multiple studies have demonstrated that CGA has various biological effects, including antioxidant [7], neuroprotective [8,9], anti-obesity [10], anti-diabetic [11], anti-inflammatory [12], antioxidant [6], antitumor [13] and radioprotective [14], *etc*. Due to the potential medicinal value, CGA has gained much attention in recent years. However, the pharmaceutical utilization of CGA is limited by its poor bioavailability. Studies [15–17] have showed that even the absorption in intestines is crucial to obtain high bioavailability, only slight amount of CGA can be absorbed through alimentary tract after oral administration. The reason is assumed to be the poor lipid-solubility. It's difficult for the water-soluble CGA to pass through the lipid-rich biomembrane, since they mainly incorporates into the hydrophilic part of membrane [18]. Thus, we aim to develop a novel chlorogenic acid-phospholipid complex to enhance the intestinal absorption thereby oral bioavailability of CGA.

The drug-phospholipid complex have been widely used to improve the oral bioavailability of various poorly absorbed herbal medicines. The interaction between drug molecules and phospholipids is the formation of hydrogen bonds and/or hydrophobic interactions. Through complexing with phospholipids, the drug tends to share some of phospholipid's amphiphilic property, thereby increasing the drug's dissolution in gastro-intestinal fluid and facilitating drug transferring through biomembrane, tissue, or cell wall. The drug-phospholipid complex is able to improve both solubility and gastrointestinal permeability of drugs. Other advantages of drug-phospholipid complexes include the increasing of stability profile and prolonging the duration of action of drugs [19]. Sauvik Bhattacharyya et al [20] have prepared CGA-PC through a simple method and suggested the protection effects of the complex against UVA induced oxidative stress produced in the rat skin. Besides, nanoparticles [21] and liposomal engineering process [22] are also reported to be able to enhance the oral bioavailability of CGA. In addition, the liposomal formulation enhances the *in vivo* antioxidant activity of CGA. However, the preparation of CGA-PC has not been optimized and few have reported the anti-inflammatory evaluation *in vivo* of CGA-PC orally administrated. Thus, we optimized the preparation process of CGA-PC, characterized and evaluated this complex. Although it has been proposed that chlorogenic acid is beneficial for cardiac inflammation, the precise mechanisms which regulate mtROS-mediated MAP kinase activation have not been elucidated. In the present study, we evaluated whether oral treatment of CGA-PC affected cytokines production and myocardial ischemia reperfusion injury in SAMP8 mice. We also investigated the MKP-1 and JNK signaling with mtROS production and oxidative stress in cardiac aging process. We found that mtROS played a critical role in post-infarction inflammatory response and addressed the relevance of JNK in CGA-PC treatment. It might be worthwhile to extend our knowledge on the *in vivo* anti-inflammatory effect of CGA by studying its role against cardiac aging related inflammatory status.

Table 1
Factor levels for the experimental design.

Factors	Levels				
	−1.68	−1	0	+1	+1.68
X_1(h)	0.32	1	2	3	3.68
X_2(°C)	23	30	40	50	57
X_3(w:w)	1.32	2	3	4	4.68

2. Materials and methods

2.1. Chemicals

CGA was purchased from Chengdu Must Bio-technology Co. Ltd, China, purity 98%. Phospholipid (Lipoid E80, purity 80%) was purchased from Shanghai Dongshang Biology Technique Ltd., China. Reference standards of CGA and puerarin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The CGA and phospholipid (at weight ratio of 1:2, 1:3, 1:4, respectively) were placed in 10 ml ethyl acetate. The reaction temperature of the complexe was controlled to 30 °C/40 °C/50 °C using water bath (DF-101S, Gongyi City Yuhua Instrument Co. Ltd, China) and was maintained at the specified temperature for a reaction time of 1/2/3 h. After completion, the mixture was filtered through 0.45 μm microporous membrane (Nylon66, Jin Teng experimental instrument factory, Tianjin, China). Then ethyl acetate was evaporated off under vacuum (RE 2000B, Yarong biochemistry instrument factory, Shanghai, China). The residue was dried, and stored in −20 °C. All the above-mentioned steps were performed under aseptic conditions. The content of CGA in phospholipids complex was determined using an HPLC method (Shimadzu, Japan), the determination condition was as follows. The stationary phase, Hypersil BDS C18 column (200mm × 4.6 mm, 5 μm, Thermo Fisher Scientific Inc., Waltham, MA, USA) was maintained at 35 °C. The mobile phase was a mixture of water (pH adjusted to 3.0 using H_3PO_4) and acetonitrile (90:10, v/v). The flow rate was 1.0 ml/min, and a wavelength of 327 nm was used for detection. CGA-PC was dissolved in HPLC mobile phase and filtered through 0.45 μm microporous membrane, then injected into the HPLC system (Shimadzu, Japan).

The yield of CGA 'present as a complex' (%) was determined using the following Eq. (1):

$$\text{The yield} = \frac{a}{b} \times 100\% \quad (1)$$

Where *a* was the content of CGA 'present as a complex' in the complex, *b* was the total reaction dosage of CGA.

2.2. Optimization via central composite design

A three-factor central composite design was applied to optimize the process. The influence at various levels of the effect of independent variables the reaction time (X_1, h), reaction temperature (X_2,°C) and drug-phospholipid ratio (X_3, w:w) on the dependent variable the yield (Y, % w/w) were systematically studied. A statistical model was used to evaluate the response employing the following Eq. (2):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_{12} + b_{22}X_{22} + b_{33}X_{32} + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (2)$$

where Y was the dependent variable, b_0 was the arithmetic mean response of the 20 runs, and bi was the estimated coefficient for the factor Xi. The level values of three factors and the composition of the central composite design batches 1–20 are shown in Tables 1 and 2.

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