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Oleuropein prevents the development of experimental autoimmune myocarditis in rats

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ABSTRACT

Oleuropein (OLE) is a natural secoiridoid that is derived from *Olea europaea*. OLE possesses cardioprotective effects in experimental models of hypertension, myocardial infarction, atherosclerosis and hyperlipidaemia. In the present study, the effects of OLE on experimental autoimmune myocarditis (EAM) were evaluated. EAM in rats were induced by subcutaneous injections of porcine cardiac myosin. Cardiac function parameters, myocardial pathology, myocardial inflammatory cell infiltration and nuclear factor kappa-B (NF-κB) expression were measured. Our data showed that the postmyocarditis rats exhibited increased left ventricular end systolic diameters, left ventricular end diastolic diameters, left ventricular end-diastolic pressures (LVEDP), and decreased ejection fractions. However, OLE significantly suppressed these changes in EAM rats. Histological analysis revealed that myosin induced miliary foci of discolouration on endocardial surfaces and extensive myocardial injuries with inflammatory cell infiltration were significantly improved by OLE therapy. A definitive positive correlation between the histological scores and LVEDP was observed. Moreover, OLE inhibited $CD4^+$, $CD8^+$ cells and macrophage infiltration in myocardium and decreased the serum production of tumour necrosis factor-a (TNF-a), interleukin-1β (IL-1β) and IL-6 in EAM rats. Expectedly, the myocardial levels of NF-κB p65, p-IκBa, IKKa were significantly attenuated by OLE, indicating the inhibitory effects of OLE on the NF-κB pathway. Furthermore, OLE decreased the myocardial expressions of phosphorylated-p38 MAPK, phosphorylated-ERK, and did not change the levels of p38 MAPK and ERK in EAM rats. Collectively, our results suggest that OLE effectively prevents the development of myocarditis, at least in part, by inhibiting the MAPKs and NF-κB mediated inflammatory responses.

1. Introduction

Myocarditis is a common cardiovascular disorder and is one of the main causes of dilated cardiomyopathy (DCM). Myocarditis is usually caused by several factors, such as viral infections, bacterial infections, drug toxicity and autoimmune reactions [1]. Currently, the treatment and prognosis of myocarditis varies based on the underlying cause. In most cases, the precipitating factor cannot be determined and only supportive treatments can be provided [2]. Unfortunately, there is no ideal treatment for myocarditis because immunosuppressants are not a widely accepted treatment option, and the application of glucocorticoids continues to be debated [2].

The mechanisms involved in myocarditis are extremely complicated and have not been fully elucidated; however, the importance of inflammatory destruction and disordered immune responses in the pathogenesis of myocarditis has been highlighted in recent investigations [3]. In myocarditis patients and experimental models, inflammatory cytokines secreted from activated immunocytes destroy the functionality of cardiomyocytes. Additionally, the inflammatory cells (T cells and macrophages) attack and impair the myocardium and magnify the cardiac inflammatory reaction, further damaging the myocardium [4–5]. Nuclear factor kappa-B (NF-κB) is one of the cross-talk points of multiple inflammatory signal pathways that play key roles in regulating the expression of cytokines [6]. Extracellular-regulated kinases (ERK) and p38 mitogen-activated protein kinases (MAPK) are activated in innate and adaptive immunity and signal via different routes to alter the translation and expression levels of various pro-inflammatory cytokines [7]. It has been previously demonstrated that NF-κB and MAPK signalling pathways are overactive in human and experimental myocarditis [8–10].

A better understanding of the inflammatory responses in the pathogenesis of myocarditis would provide new tools to treat the disease. In recent years, anti-inflammatory therapy has become a promising strategy. Much of the attention has been focused on naturally occurring terpene compounds, because most of them are beneficial for cardiovascular disease prevention. Oleuropein (OLE) is a popular

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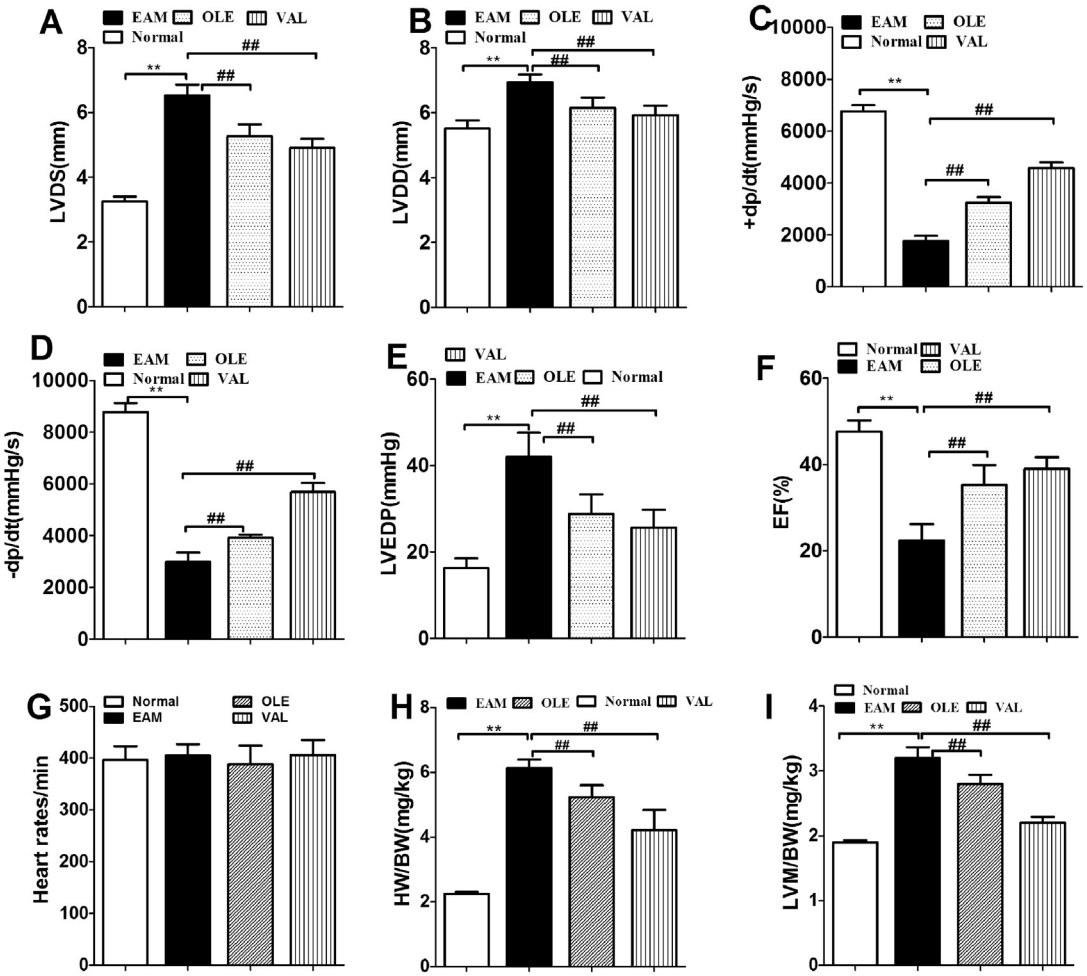

Fig. 1. OLE treatments improved the parameters of cardiac functions in EAM rats. A: LVDS; B: LVDD; C: + dp/dt; D: − dp/dt; E: LVEDP; F: EF; G: heart rate; H and I: HMI and LVMI. Values are presented as the mean ± SD, n = 7–9. $^{**}P < 0.01$ vs. normal; $^{\#\#}P < 0.01$ vs. EAM.

secoiridoid derived from *Olea europaea* and has been previously reported to exert numerous pharmacological benefits, including anti-bacterial, anti-viral, antioxidant and anti-tumour activities [11]. OLE intake effectively induces hypotensive and lipid-lowering effects and prevents atherosclerosis and cardiac remodelling after myocardial infarction [12–14]. Interestingly, when OLE was used for preventing cardiovascular illness, it was found to have immunomodulatory effects, as shown by the decreased production of inflammatory cytokines and reduced inflammatory cell infiltration in various models [15–18]. Despite many reports that have demonstrated the beneficial effects of OLE in cardiovascular diseases, its efficacy in myocarditis remains unknown. Therefore, the purpose of the present study was mainly to examine the effects of OLE in an experimental autoimmune myocarditis (EAM) model.

2. Materials and methods

2.1. Materials

OLE (purity > 90%, endotoxin free) was obtained from XiAn Bella Biotechnology Co., Ltd. (XiAn, China). Valsartan (VAL, purity > 90%) was purchased from Aladdin Biotechnology Co., Ltd. (Shanghai, China). MTT and porcine cardiac myosin were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Mycobacterium tuberculosis H37Ra was purchased from Difco Laboratories (Detroit, MI, USA). The antibodies against CD3, CD4 and CD8 were obtained from BioLegend (San Diego, CA), and antibodies against ED-1, NF-κB p65, IκBa, p-IκBa, IKKa, p38 MAPK, ERK, phosphor-p38 MAPK (p-p38 MAPK) and p-ERK were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). The ELISA kits for measuring tumour necrosis factor-a (TNF-a), interleukin-1β (IL-1β) and IL-6 were purchased from R & D Systems (Minneapolis, USA).

2.2. Animals

Fifty-two, healthy, adult male Lewis rats (8 weeks old) were purchased from the Beijing Vital River Lab Animal Technology Co., Ltd. (Beijing, China). All rats were kept in the animal house under controlled conditions of 25 ± 1 °C, relative humidity of 60 ± 5% and a light–dark cycle of 12 h: 12 h. The rats were provided with regular chow and water ad libitum. Institutional and national guidelines for the care and use of laboratory animals were followed. This study was approved by the Ethics Committee on Animal Care and Use of the Hang Zhou Red Cross hospital.

2.3. EAM model and treatments

EAM in Lewis rats was induced by immunization with porcine cardiac myosin according to previously reported methods [19]. Briefly, 0.1 ml of porcine cardiac myosin (10 mg/ml) mixed with 0.1 ml Freund's complete adjuvant (FCA) supplemented with mycobacterium tuberculosis H37Ra was injected subcutaneously into the foot pads of rats on days 0 and 7. The rats in the control group were immunized with only FCA in the same manner. After 4 weeks of the first immunization,