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Original article

Inhibitory effect of alpinate Oxyphyllae fructus extracts on Ang II-induced cardiac pathological remodeling-related pathways in H9c2 cardiomyoblast cells

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ABSTRACT

Background: Our previous studies have demonstrated that Ang II induced IGF-II and IGF-II R via ERK and JNK signaling pathways and further induces cardiac cell apoptosis.
Purpose: The present study investigates the protective role of alpinate Oxyphyllae fructus (AOF; *Alpinia oxyphylla* Miq) extracts on angiotensin II (Ang II)-stimulated H9c2 cardiomyoblast cells.
Methods: Western blotting was used to analyze the molecular mechanism involved in Ang II-treated H9c2 cells.
Results: AOF inhibits cardiac hypertrophy, apoptosis, mitochondrial dysfunction, and cardiac remodeling in Ang II-treated H9c2 cells.
Conclusion: All these data collectively suggest us that, AOF significantly inhibits Ang-II induced H9c2 cells apoptosis by suppressing the mitochondrial apoptotic pathway.

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1. Introduction

With a constant increase in life expectancy, the elderly population is expanding rapidly (conventionally, a cutoff point for advanced age in humans may be set at 65 years). The magnitude of the problem arising from this factor was emphasized in a recent report stating that individuals normotensive at 55 years of age have a 90% lifetime risk of developing hypertension [1]. Therefore, aging is often considered a significant risk for cardiac disease [2]. In the elderly, systolic blood pressure increases because of the arterial stiffness produced by structural alterations of arterial walls that occur with aging [3]. Consequently, aging poses major health concerns, and quite commonly contributes to cardiovascular morbidity and mortality via severe heart damage.

Angiotensin II (Ang II) plays an important role in cardiovascular diseases, for example, hypertension, atherosclerosis, left ventricular hypertrophy (LVH), and heart failure [4–10]. Most studies demonstrate that Ang II induces cardiovascular hypertrophy, cardiac apoptosis, mitochondrial dysfunction, and cardiac remodeling through activation of the Ang II type 1 receptor (AT1R) [4,7,11,12]. However, the opposite theory indicates AT2R causes opposite effects, for example, cardiac growth-promoting effects [13–17]. Although the functions of both major Ang II receptors prove ambiguous, the harm caused by Ang II to cardiomyocytes is beyond question [10,18–22]. In cardiac hypertrophy, calcium-dependent phosphatase calcineurin dephosphorylates the nuclear factor of activated T cells 3 (NFAT3) transcription factor, allowing it to translocate to the nucleus, after which atrial natriuretic (ANP) and b-type natriuretic peptide (BNP) are overexpressed. Gαq signaling and the mitochondrial membrane potential play vital roles in cardiomyocyte apoptosis [23].

Insulin-like growth factor II (IGF-II) also stimulates myocardial hypertrophy, apoptosis, and remodeling [24–27]. Our prior study demonstrated that Ang II seems to induce IGF-II and IGF-II R via extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK) signaling pathways, respectively, and further activates cardiac cell apoptosis through calcineurin-dependent pathways [28], ultimately causing heart failure.

Alpinate Oxyphyllae fructus (AOF; *Alpinia oxyphylla* Miq) ranks among the most important traditional Chinese medicines and has been used to treat diarrhea, polyuria, ulceration, dementia, tumors, and gastralgia, according to Chinese Pharmacopoeia [29]. Several experiments indicate its potential as a neuroprotective agent, both in water and ethanol extracts [29–33]. In Korea, AOF serves as a medicinal plant, also used to treat various symptoms accompanying hypertension and cerebrovascular disorders [30].

Methanol extract of AOF reportedly has cardiotonic effectiveness [34]; whether it has protective and rescue effects on Ang II-stimulated H9c2 cardiomyocytes remains unknown. This study evaluates the pathophysiological mechanisms of AOF in cardiac hypertrophy, apoptosis, and the mitochondrial dysfunction and remodeling induced by Ang II treatment in cardiomyoblast H9c2 cells.

2. Materials and methods

2.1. AOF extraction

We purchased AOF in fragmented form from Shin-Long Pharmaceutical Company (Taichung, Taiwan); 150 g of AOF fragment was extracted with 600 mL of boiling water for 2 hours. The filtrate was concentrated at reduced pressure for convenience. The extract solution was stored at 4°C and spray-dried to yield a powdered extract.

2.2. Cell culture

H9c2 cardiomyoblasts from the American Type Culture Collection (ATCC, CRL-1446, Rockville, MD, USA) were cultured in 100-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 μg/mL penicillin, 100 μg/mL streptomycin, 2 mM glutamine, 1 mM HEPS buffer, and 10% fetal bovine serum in humidified air (5% CO_2) at 37°C. H9c2 cells were incubated in serum-free essential medium for 4 hours before treatment with the indicated agents.

2.3. Immunoblotting

To isolate total proteins, cultured myocardiac cells were washed with cold phosphate-buffered saline (PBS) and resuspended in lysis buffer [50 mM Tris, pH 7.5, 0.5 M NaCl, 1.0 mM EDTA, pH 7.5, 10% glycerol, 1 mM B-Mercaptoethanol (BME), 1% octylphenyl-polyethylene glycol (IGEPAL-630), and proteinase inhibitor cocktail (Roche Molecular Biochemicals, IN, USA)]. After 30 minutes incubation on ice, the supernatant was collected by centrifugation at 12,000 rpm for 30 minutes at 4°C. The protein concentration was determined by the Bradford method. Samples with equal proteins (35 μg) were loaded and analyzed by Western blotting. Briefly, proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Belfor, MA, USA). Membranes were

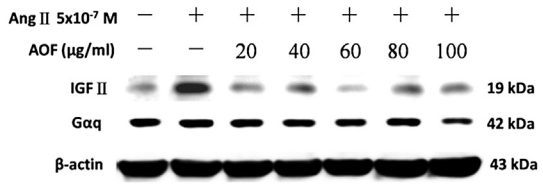

Fig. 1 − The effect of AOF on Ang II-induced IGF-II/II R signaling in H9c2 cells. H9c2 cells at 80% confluence were treated with Ang II (5×10^{-7} M) for 1 hour, then administered AOF (20, 40, 60, 80, and 100 μg/mL) for a further 23 hours. The total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and immunoblotted with antibodies against IGF-II and Gαq proteins. Equal loading was assessed with anti-β-actin antibody. Cell culture without treatment served as control. Ang II = angiotensin II; AOF = alpinate Oxyphyllae fructus; IGF = insulin-like growth factor II; PVDF = polyvinylidene fluoride; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.