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Abstract of thesis

In chapter 1, the author describes the general introduction of this thesis including background and objective of this study. Cardiovascular diseases (CVDs) include a broad category of disease such as coronary artery disease, stroke, heart failure, arrhythmia, and other heart diseases, and are the leading cause of death worldwide, accounting for 32% of all death. Cardiac fibrosis is defined as the imbalance of extracellular matrix (ECM) metabolism and is related to cardiac dysfunction in many CVDs. Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, leading to morbidity and mortality due to complications such as stroke and heart failure. Electronic remodeling (i.e., ion channel abnormalities) and structural remodeling (i.e., fibrosis) of the atria act as a trigger for initiation of AF and a proarrhythmic substrate that sustains the arrhythmia. Furthermore, one risk factor of AF is a hypertension, which is closely associated with elevation of angiotensin II (AgII) level. In electrical remodeling, the ablation surgery suppresses the abnormal excitation that becomes the trigger of AF, and there are antiarrhythmic drug treatments that target the ion channel, called downstream therapy. On the other hand, treatment that suppresses structural remodeling of AF is called upstream therapy.

Isorhamnetin (ISO) is a natural-derived flavonoid found in several plants and plant-derived foods. ISO is a methylated form of quercetin and is suggested to be an intermediate metabolite of quercetin after absorption. Therefore, it is important to understand the biological function and molecular mechanism of ISO as a mediator of the

beneficial effects of quercetin. A number of studies reported various biological activities of ISO, including anti-inflammatory, anti-oxidant, and anti-adipogenic effects. Previous study found that ISO alleviated steatosis and fibrosis in a nonalcoholic steatohepatitis mouse model by reducing the expression of liver injury marker, transforming growth factor β (TGFβ), and the fibrogenic marker collagen type I A I (Col1A1). However, little is known about the protective effect of ISO against cardiac fibrosis. In this thesis, the author aims to clarify the potential of ISO on treatment of AF and its molecular mechanism.

In chapter 2, the author investigated cardioprotective effect of ISO using *in vivo* and *in vitro* models. The author initially performed the global gene analysis using human aortic endothelial cells (hAECs) to examine the effect of ISO on the transcription of fibrosis-related genes. A number of genes related to myocardial development and fibrosis were downregulated by the treatment of ISO, suggesting that ISO has the potential to suppress the fibrosis and myocardial development. Next, the author tested the effect of ISO against AgII induced structural abnormalities in the heart using *in vivo* model. One week before the implantation of the mini-osmotic pumps, mice were administered with ISO intraperitoneally every day for three weeks. Then, echocardiography, histological examination and biochemical assays were performed. The echocardiographic analysis showed that the interventricular septum (IVS) and left ventricular posterior wall (LVPW) thickness were enlarged in AgII-infused mice in both systole and diastole, but the enlarged IVS and LVPW thickness were markedly reduced in ISO-treated mice. The LV wall was extended in AgII-infused group and recovered in ISO treat group. Furthermore, LV mass was markedly enlarged in the AgII infused mice, but ISO nullify the enlargement of LV mass caused by AgII. These results suggested that ISO reversed AgII-induced enlargement of ventricular in model mice. Histological analysis revealed that the pathological abnormalities induced by AgII were significantly reduced by ISO treatment in mice, suggesting that ISO inhibits morphological aberrations of heart tissues caused by AgII *in vivo*. Moreover, the expressions of pro-inflammatory and pro-fibrogenic marker genes caused by AgII in heart tissue were measured by RT-qPCR. ISO significantly suppressed the upregulations of *Tgfβ1*, *Tgfβ2*, *Col1a1*, and *Nppb*.

In chapter 3, the author addressed the effect of ISO against AgII-induced AF. The pathophysiological mechanism of AF is explained by electrical remodeling and structural remodeling. In a process of electrical remodeling, phosphorylation of ryanodine receptor 2 (RyR2) triggers the leak of Ca^{2+} to sarcoplasmic reticulum (SR). The author tested the effect of ISO on AF by using an electrophysiological analysis. Compared with control, AF inducibility was dramatically increased by AgII and significantly decreased by ISO. AF duration was also remarkably prolonged by AgII and significantly reduced by ISO. Atrial effective refractory period (A-ERP) was reduced by AgII and recovered by ISO. Incidences of diastolic intracellular Ca^{2+} abnormal activities (SR Ca^{2+} leakage) were observed in AgII group, whereas ISO treatment eliminated these abnormalities. Also, in HL-1 cells, cardiac muscle cell line, action potential duration (APD) was significantly prolonged and delayed afterdepolarizations (DADs) were enhanced in AgII-exposed HL-1 cells. The lengthening APD was restored to normal duration, and DADs were diminished by treatment of ISO. These results suggested that ISO could alleviate AgII-induced electrophysiological abnormalities. The author also conducted histological examination to clarify the effect of ISO on pathological fibrosis. Compared to control group, severe disorganization of myofibrillar arrays, and cytoplasmic vacuolization and infiltration with neutrophil granulocytes were observed in AgII-treated mice. ISO pretreatment remarkably reduced these pathological abnormalities. The ratio of heart weight/body weight (HW/BW) and atrium weight/body weight (AW/BW) were

significantly increased by AgII, while ISO treatment reduced the increase in HW/BW and AW/BW. Additionally, ISO reduced the AgII-mediated increase in cardiomyocyte size in left ventricular. These results demonstrated that ISO reversed the AgII-induced morphological abnormalities in cardiomyocytes in mice. Furthermore, ISO significantly reversed increased protein expressions of Nf-κB and transient receptor potential (TRPC), and attenuate phosphorylation of RyR2, suggesting that ISO prevented AgII-induced overexpression of hypertrophy, inflammatory and fibrogenic markers in mice.

In chapter 4, the author describes the general discussion to state the overall mechanism of ISO. One of the reasons for the suppression of AF vulnerability is the suppression of electrical remodeling due to the suppression of the occurrence of diastolic SR Ca^{2+} leak by abrogating CaMKII activation and phosphorylation of RyR2 in serine 2814 site. Related to this, ISO inhibited the generation of DADs by spontaneous Ca^{2+} release by decreasing the Ca^{2+} sensitivity of RyR2 by inhibiting the activation of CaMKII. Also, ISO restored AP morphology by suppressing the overexpression of CaMKII and Cav1.2, which are involved in AP morphology. Furthermore, the author discussed that the inhibitory effect of ISO on structural remodeling was another factor responsible for its antiarrhythmic effect. ISO inhibited the activation of JNK and ERK, which are mediators of MAPKs that lead to ROS production, fibrosis, and hypertrophy. ISO treatment also suppressed the overexpression of TRPC channels, which regulate intracellular Ca^{2+} input through the response of cardiac fibroblasts.

Abstract of assessment result

【Review】

In the past, steroids, HMG-CoA reductase inhibitors, and angiotensin II receptor blockers (ARB) have been recognized as upstream therapies for AF treatment. However, these drugs have many limitations, including adverse effects and limited efficacy. Thus, preventive therapy for AF has not been completely established. Therefore, there is an urgent need for the discovery of effective compounds or substances with fewer side effects. To date, many papers have shown that ISO inhibits fibrosis and hypertrophy, and related diseases have been studied in various organs. In this thesis, the applicant clearly shows the mechanism by which flavonoids suppress atrial fibrillation. There have been no report describing the function of flavonoid for the inhibition of the development of AF by modulating both structural and electrical remodeling of the atria. Of particular interest and novelty is that flavonoids inhibit calcium leakage from RyR2 receptors by suppressing the activation of CAMKII, which is responsible for calcium handling and is believed to be the cause of atrial fibrillation. Given the current skepticism regarding existing preventive drugs and the great interest in the development of novel substances, this study could have important therapeutic implications. Furthermore, the results of this study should facilitate and accelerate investigations of the therapeutic effects of other natural compounds on AF, which contributes to the field of food innovation.

【Result】

The final examination committee conducted a meeting as a final examination on 18/1/2022. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Food Innovation.