Topic: 3. Heart Failure and Cardiomyopathy

The Inhibitory Effects of Curcumin Glucuronide on p300-HAT Activity and Hypertrophic Phenylephrine-Induced Responses in Cardiomyocytes

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Introduction and Objectives

Hemodynamic stimuli such as hypertension and myocardial infarction activate an intracellular signaling pathway, finally reach the nuclei of cardiomyocytes, change the pattern of hypertrophic response gene expressions, and induced cardiac hypertrophy and systolic dysfunction. These eventually lead to the development of heart failure. In our study, we demonstrated that an intrinsic histone acetyltransferase, p300, is a critical role on hypertrophic response in cardiomyocytes and may be a pharmacological target for heart failure therapy. It has been reported that several compounds inhibit HAT activity *in vitro* and in culture. One of them, a natural compound curcumin (CUR) isolated from *Curcuma longa* has a p300-specific HAT inhibitory activity and cell permeability. Previous study showed that we demonstrated that CUR significantly prevented the development of heart failure by inhibiting p300-HAT activity in two different heart failure model rats. In an oral administration, CUR almost undergoes rapid metabolism by glucuronidation and exists in blood as curcumin glucuronide (CUR-G). So we hypothesized that CUR-G itself could exhibit beneficial biological activities as well as CUR. The purpose of this study was to investigate whether CUR-G inhibited p300-HAT activity *in vitro* and hypertrophic responses in cardiomyocytes.

Materials and Methods

First, to compare the inhibitory effects of CUR and CUR-G against p300-HAT activity, we performed an *in vitro* HAT assay using a recombinant p300-HAT domain and core histones. The activity of p300-HAT was quantified by immunoblotting and the ratio of acetylated histone-H3K9 to total histone-H3 was calculated. Next, primary cardiomyocytes from

neonatal rats in culture were stimulated with 30 μ M phenylephrine (PE), an α 1-adrenergic agonist, in the presence or absence of CUR or CUR-G for 48 hours. Protein extracts from these cell were subjected to immunoblotting. The mRNA levels of hypertrophic response genes, such as atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) were quantified by RT-PCR. Cardiomyocytes were stained with anti-cardiac MHC antibody and cell surface area was measured.

Results

The results of *in vitro* HAT assay showed that the half maximal inhibitory concentration (IC_{50}) value of CUR-G on p300-HAT activity was 37.3 μ M, while that of CUR was 9.4 μ M. In cardiomyocytes, 30, 100 μ M of CUR-G, but not 10 μ M of CUR-G, significantly inhibited PE-induced histone-H3K9 acetylation whereas 10 μ M of CUR suppressed this acetylation. Moreover, in cultured cardiomyocytes, 100 μ M, but not 10 μ M of CUR-G, significantly repressed PE-induced hypertrophic response gene activations such as ANF and BNP and increases in cardiomyocyte size and myofibrillar organization.

Conclusions

These results indicate that CUR-G has p300-HAT inhibitory activity and prevented PE-induced hypertrophic responses that effects are lower than those of CUR. The reduction of these effects may be due to the depression of the inhibitory activity against p300-HAT by the structure of glucuronic acid conjugated to CUR. These findings may help to elucidate the therapeutic potency of CUR. Further studies, especially *in vivo* studies, are needed to better understand the pharmacokinetics of CUR and CUR-G. ■

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