A NOVEL NON-ACTIVE SITE INHIBITION OF CARBONIC ANHYDRASE BY ACACETIN: IMPLICATIONS FOR CARDIOPROTECTION

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Abstract

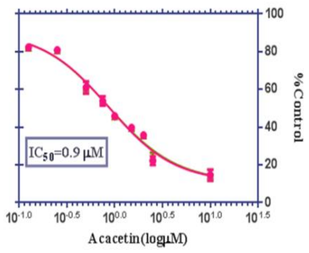
It is a well established fact that increased levels of Carbonic Anhydrase (CA) is a prognostic molecular marker of Cardiac Hypertrophy(CH), and that inhibition of CA prevents and even reverts this disease state. The present study reports the identification of acacetin, a partial methylated flavone, which inhibits both the expression as well as the activity of CA II (IC_{50} = 0.9μM). Acacetin binds to a novel 'non-active site' region of CA II and disrupts the nucleophilicity of the active site in a very unique manner. Additionally, Acacetin also inhibited the intracellular CA activity in H9c2 rat embryonic myocardial cells, which further decreased the intracellular calcium levels, resulting in prevention of the mitochondrial membrane depolarization. By inhibiting this hypertrophic cascade that plays a critical role in CH, Acacetin could thus play a significant role in the reversal of CH.

Materials & Methods

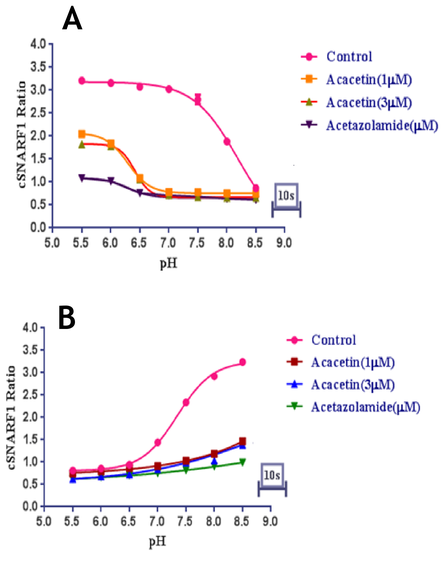
Measurement of carbonic anhydrase II activity, intracellular carbonic anhydrase activity, intracellular calcium, mitochondrial membrane potential, RT-PCR and *insilico* docking.

Results

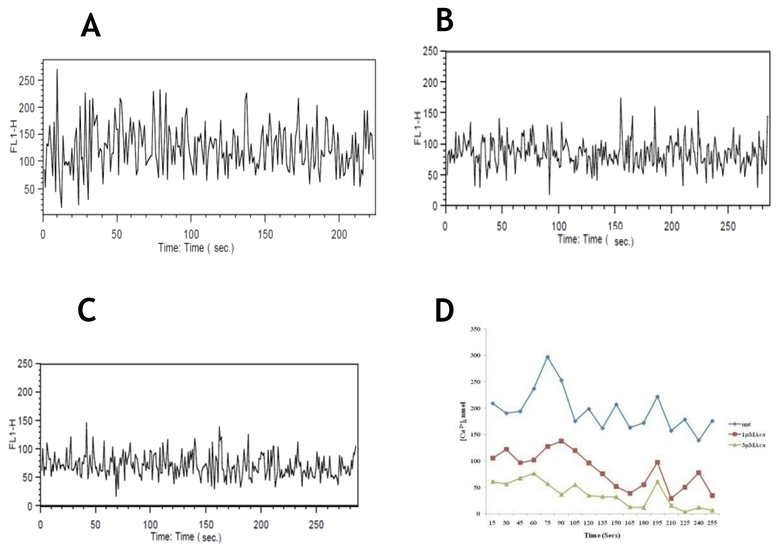
1.IC_{50} Curve Of Acacetin For Human CA II Isozyme inhibition



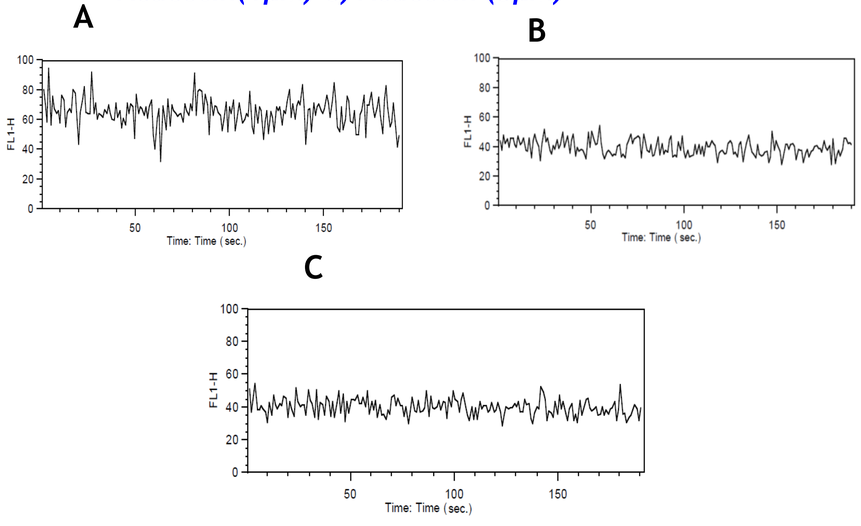
2.Intracellular carbonic anhydrase activity: Determining cytoplasmic CO_2 hydration rate from pH_i dynamics in intact H9c2 cells. Variations in pH_i at 37℃ in A) 5% CO_2 buffered solution to B) Hepes-buffered solution (CO_2 free).



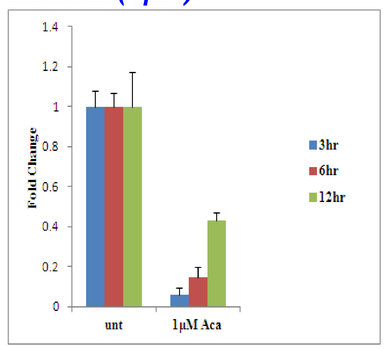
3. Intracellular calcium levels in H9c2 cells with Fluo-3-AM A) Control B) Acacetin(1μM) C) Acacetin (3μM) D) $[Ca^{2+}]i$ curves for control, acacetin(1μM& 3μM)



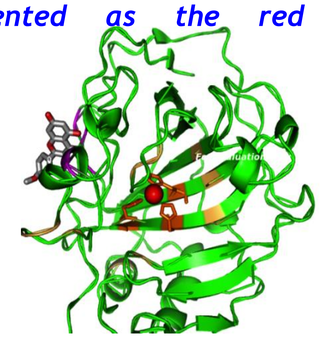
4. Rapid acidification on exposure 5% CO_2 buffered solution causes depolarization of Mitochondrial membrane potential in H9c2 cells A) Control B) Acacetin(1μM) C) Acacetin (3μM)



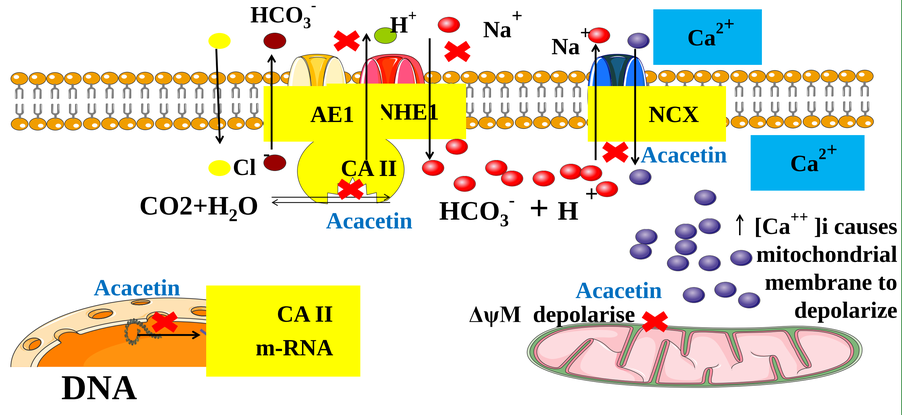
5. Determining CA II gene expression levels in intact H9c2 cells in Control & Acacetin(1μM)



6. Interaction of acacetin(grey) with CA II. The protein is represented as a green ribbon, the catalytic Zn^{2+} ion is represented as the red sphere,



7. Integrative model representing the role of CA II in cardiac hypertrophy adapted from Alvarez et al . ✖ in the figure indicates the inhibition of CA II by acacetin and thus suggesting a potential mechanism to revert cardiomyocyte hypertrophy.



Conclusion

Thus, acacetin a partially methylated flavone inhibits CA II by a novel non-active site binding mechanism. Also it inhibits the hypertrophic cascade in H9c2 cells and significant reversal of cardiac hypertrophy.

References:

B. V Alvarez, A.L. Quon, J. Mullen, J.R. Casey, Quantification of carbonic anhydrase gene expression in ventricle of hypertrophic and failing human heart., BMC Cardiovascular Disorders. 13 (2013) 2. doi:10.1186/1471-2261-13-2.