(R/S)-17 Hydroxyeicosatetraenoic acid (HETE) induces the expression of CYP1B1 at the transcriptional level and increases its catalytic activity

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Introduction

Cardiac hypertrophy is a response to initial decreased cardiac pumping or pressure and volume overload (1).

Previous studies conducted in our laboratory reported that cytochrome P450 1B1 (CYP1B1)-mediated arachidonic acid metabolites called midchain hydroxyeicosatetraenoic acids (HETEs) have a cardiotoxic effect and are involved in the development of cardiac hypertrophy (2).

Hypothesis

(R/S) 17-HETE induces CYP1B1 gene expression and plays a role in the development of cardiac hypertrophy.

Objectives

- To characterize the effect of 17-HETE enantiomers in the development of cardiac hypertrophy using adult human cardiomyocyte cells (AC16**).**
- To investigate the effect of (R) and (S)-enantiomers of 17-HETE on CYP1B1 in AC16 cells**.**

Methods

AC16 cells were treated with increasing concentrations of (S) and (R)-17 HETE for 24 hours. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR), western blot, ethoxyresorufin-O-deethyllase (EROD) assay, and Microscopic examination were conducted to determine the effect of (R/S)-17 HETE on CYP 1B1 and the cardiac hypertrophy.

Results

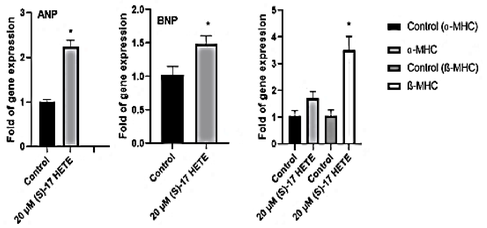

Figure 1: Effect of 20 μM (S)-17-HETE on mRNA levels of hypertrophic markers in AC16 cells.

AC16 cells were treated with (S)-17-HETE for 24h and a RT-PCR test was conducted. 20 μM (S)-17 significantly increased the mRNA level of ANP, BNP, and β-MHC. *P<0.05 compared to control. a-MHC, alpha-myosin heavy-chain; ANP, atrial natriuretic peptide; b-MHC, beta-myosin heavy-chain; BNP, brain natriuretic peptide

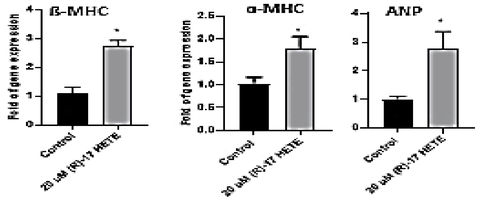

Figure 2:. Effect of 20 μM (R)-17-HETE on mRNA levels of hypertrophic markers in AC16 cells

AC16 cells were treated with (R)-17)-HETE for 24h and a PCR test was conducted. 20 μM (R)-17-HETE significantly increased the mRNA level of ANP,α-MHC, and β-MHC. *P<0.05 compared to control. a-MHC, alpha-myosin heavy-chain; ANP, atrial natriuretic peptide; b-MHC, beta-myosin heavy-chain.

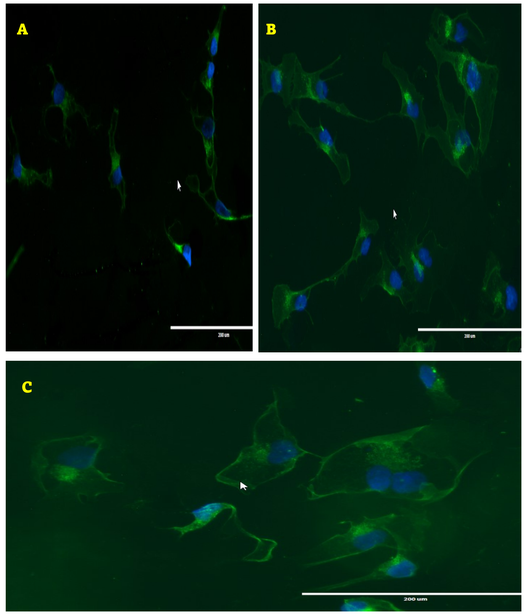

Figure 3: Effect of 20 μM (R/S)-17-HETE treatment on cell size in AC16 cells.

AC16 cells were treated with 20 μM (R/S)-17 HETE for 24h and the cells were examined under a microscope. A preliminary test has revealed a trend of size difference in AC16 cells with and without the treatment. 20 μM (R/S)-17 HETE resulted in a trend of a bigger cell size compared to the control cells.

A, AC16 control cells; **B**, AC16 cells treated with 20 μM (R)-17-HETE; **C**, AC16 cells treated with 20 μM (S)-17-HETE

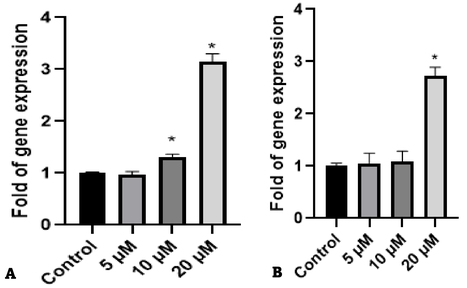

Figure 4: Effect of (S/R)-17-HETE on CYP1B1 mRNA expression.

AC16 cells were treated with an increasing concentration of (R/S) 17-HETE for 24h. A RT-PCR test was performed**. A,** 10μM and 20 μM (S)-17-HETE induced m-RNA of CYPIBI significantly (20 μM (S)-17-HETE induced CYP1B1 mRNA by 3-fold). **B**, 20 μM (R)- induced m-RNA of CYPIBI by >2.5 fold. *P<0.05 compared to control.

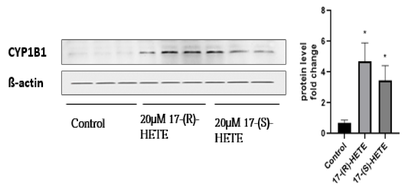

Figure 5: Effect of (S/R)-17-HETE on CYP1B1 protein expression.

AC16 cells were treated with 20 μM (R/S)-17 -HETE for 24h and western blot analysis was conducted. 20 μM (R/S)-17-HETE significantly increased the CYP1B1 protein expression. *P<0.05 compared to control.

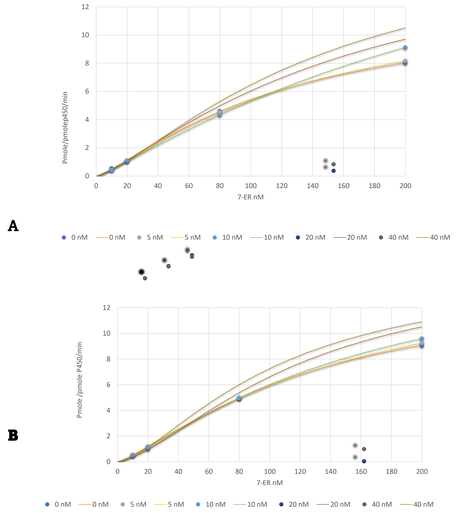

Figure 6: Effect of (S)-17 HETE (A) and (R)-17 HETE (B) on catalytic activity of recombinant CYP1B1.

We measured the O-dealkylation rate of 7-ethoxyresorufin in the presence and the absence of 17-(R/S)-HETE. Addition of 17-(R/S)-HETE to the mixture has increased resorufin formation rate mediated by human recombinant CYP1B1 enzyme

17(S)-HETE	Control	5 nM	10 nM	20 nM	40 nM
Vmax (Pmol/Pmol P450/min)	11.6647	12.7767	21.7431	17.0236	17.284
km (nM)	110.81	128.185	264.17	159.995	145.37
n	1.33045	1.28368	1.16398	1.27361	1.3742
17(R)-HETE	**Control**	**5 nM**	**10 nM**	**20 nM**	**40 nM**
Vmax (Pmol/Pmol P450/min)	13.174	15.9404	16.7167	15.2422	14.948
km (nM)	114.946	153.702	157.922	118.812	103.96
n	1.41944	1.23043	1.26117	1.53588	1.5156

Table:1 Calculated Vmax, n, and km for (S/R)-17 HETE

Vmax, maximum velocity of the reaction; km, the concentration of substrate which ½ of Vmax is achieved; n, Hill coefficient.

Summary & Conclusion

- (R/S) 17-HETE has significantly increased the expression of hypertrophic markers, as well as the cell size
- (S/R)-17-HETE increased the transcription of CYP1B1 at mRNA & protein levels significantly.
- (S/R)-17-HETE caused allosteric activation of the catalytic site of human recombinant CYP1B1 enzyme.

Future direction

- Studying the mechanism by which CYP1B1 activity is increased by 17- HETE enantiomers
- Assessing the effect of (R/S)-17 HETE in vivo in different sex

Acknowledge

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References

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